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THE PROBLEM OF THE BLAST DISEASE OF RICE*

By T. S. Sadasivan, F.A.Sc.

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Notwithstanding all that has been said and done in the field of blast disease of rice caused by the fungus *Piricularia oryzæ* Cav. in India and abroad, mainly in Japan, much more still remains to be experimented upon and clarified regarding the pathogen itself and the biologics of its parasitism.

One need hardly go into much detail of the symptomatology of this disease as it has been recently described adequately (Suryanarayanan, 1956). The most important symptoms are the typical spindle-shaped or eye-spot lesions on the leaves and the blackening of the necks of the panicle. The latter is the most destructive since the flow of nutrients into the grains is impeded resulting in a chaffy ear-head. No critical assessment of the damage seems to have been made although it is generally agreed by many workers that it is of sufficient magnitude. The most feasible method of combating the disease has been one of breeding resistant types but, not infrequently, break-down of resistance has been noted. This is not surprising because several physiological races of the fungus have been identified and host susceptibility is determined not only by genetic factors but also, to a large extent, by a set of very critical environmental factors, particularly the nycto-temperature which influences the metabolic pattern of the host (Suryanarayanan, 1958 *a*). This contribution of Suryanarayanan in ascribing the incidence of the disease to low night temperatures has opened up a new field for investigation and explains many of the discrepancies noted in the disease proneness of the host in the field, in India and elsewhere. Indeed, micro-climatology in relation to the blast disease is still a matter for future investigation. Besides, our earlier investigations on the physiology of the pathogen have revealed the essentiality of Fe, Mn, Zn and possibly Cu as well as the vitamins thiamine and biotin for both growth and sporulation *in vitro*. Even though it is not possible to give an isolated consideration to these micro-nutrients *in vivo*, it is, nevertheless, plausible that these micro-nutrients as well as other macro-nutrients like amino acids, amides, sugars and the like are available on the leaf surface being exuded as guttates or reaching the cuticle by the process of exosmosis (Subba-Rao

* Presidential address delivered on the 29th December 1958 to Section 'B' of the 24th Annual Session of the Indian Academy of Sciences, Baroda.

and Suryanarayanan, 1957). In view of the foregoing it is obvious that the problem of the blast disease of rice is one which calls for attention to the intimate host-parasite relationships for adopting any effective control measures.

In light of evidences obtained in our laboratory and elsewhere, *P. oryzæ* appears to have more specialized metabolic requirements than at one time imagined. For instance, *P. oryzæ* is heterotrophic to vitamins of the B group, *viz.*, thiamine and biotin (Suryanarayanan, 1958 *b*). The intact thiamine molecule, however, is not needed on a sucrose-nitrate medium, the pyrimidine portion of the vitamin alone being fully effective indicating the capacity of the organism to synthesize the thiazole fraction. This ability of *P. oryzæ* to grow with pyrimidine alone is evident only after 6 days, for growth in the earlier stages is apparent only when both thiazole and pyrimidine are present. Another curious feature about the biosynthesis of thiamine by *P. oryzæ* is that the synthesis of the thiamine molecule by the fungus appears to depend on the type of substrate sugar (Suryanarayanan, 1958 *c*). Pyrimidine which is equivalent to the full molecule of thiamine when the substrate sugar is sucrose or maltose is not fully effective when the carbon source happens to be glucose. This is rather surprising since it is generally believed that a disaccharide like maltose has to be first enzymatically hydrolyzed and the products in this case would be glucose only. We, therefore, consider that pyrimidine is fully effective only when supplied with the γ-form of glucose which comes into existence when disaccharides are hydrolyzed, though temporarily. Further, the essentiality of Fe, Mn, Zn and to a certain extent Cu for this fungus has been established by us and that both the heavy metals and vitamins are indispensable for growth and sporulation in culture (Appa-Rao *et al.*, 1955). Higher concentrations of the vitamins and heavy metals, especially Cu and Mn, are required for sporulation than for growth (Appa-Rao, 1956 *a*). That sporulation of the organism is dependent on a low initial concentration of the sugar and does not occur until the medium is depleted of the carbon source has also been proved (Appa-Rao, 1956 *a*).

Another interesting aspect of the behaviour of the pathogen in pure culture is its ability to grow with inorganic NO_3 nitrogen but not with NH_4 nitrogen. This is contrary to the general belief that all nitrate users should be able to use ammonia inasmuch as NO_3 is supposed to be reduced to NH_3 before the primary amino acids are built up. The failure of *P. oryzæ* to grow with inorganic ammoniacal sources of nitrogen as $(NH_4)_2SO_4$ has been traced to the concomitant development of high physiological acidity con-

sequent to accumulation of anions in the medium as the ammonium ions are rapidly absorbed (Appa-Rao, 1956 *a*, *b*). Should such a medium be buffered with $CaCO_3$ or if certain organic acids of the Kreb's citric acid cycle such as succinic, fumaric or citric acids are added in small amounts normal growth of the fungus is observed with ammonium nitrogen. This beneficial effect of the organic acids is considered to be two-fold. They either act directly as buffers or enter into the metabolic cycle to combine with the accumulated ammonium ions to form the primary amino acids and thereby reduce ammonia toxicity. While normal growth of *P. oryzæ* with weakly ionized ammonium salts, like ammonium carbonate does, indeed, favour the former view, its favourable response to ammonium phosphate appears to be one of corroborating the latter hypothesis especially in light of our recent findings on the role of phosphorus in inorganic ammonium utilization by other fungi like *Sclerotium oryzæ* Catt. and *Colletotrichum capsici* (Syd.) Butl. and Bisby.

Coming to the question of host physiology in relation to disease-resistance and susceptibility it has been our experience and of other investigators that infection often fails to develop even under optimal conditions of temperature (24–26° C.) and humidity (above 95%). The failure of the normal suscepts to take infection not only involves considerable loss of time in evaluation of disease-resistance in various strains of rice bred for the purpose but also presents a stumbling block in the evaluation of host-parasite mechanisms. Recently we have been able to pin down this elusive problem to the effect of a low nycto-temperature (about 20° C.) on the nitrogen metabolism of the host (Suryanarayanan, 1958 *a*). The mechanism of action is the ease with which NO_3 is reduced at a low nycto-temperature than at a high temperature and the subsequent shift of reactions more in favour of synthesis of soluble nitrogenous products like the amides and the amino acids. In this connection the glutamine synthesis by the rice plant under a heavy dressing of $(NH_4)_2SO_4$ is worthy of mention. Under heavy nitrogenous manuring and low nycto-temperatures much glutamine is synthesised by the host and is actually exuded in the guttates. The guttate, when it dries over the leaf surface leaves easily identifiable macroscopic crystals mainly of glutamine (identified chromatographically) which markedly stimulates the germination of *Piricularia* spores at the infection court. This stimulation by glutamine is, however, apparent only when the atmospheric temperature is about 24–26° C., which range has been established by many workers to be the optimum for successful infection of rice by the blast fungus (Suryanarayanan, 1958 *d*).

Obviously, the pattern of nitrogen metabolism of the rice plant appears to be the most important single factor connected with its genetic constitution as well as with critical environmental factors, chiefly a low nycto-temperature. It is relevant to mention here some of our early results on the nitrogen metabolism of the host which only corroborates our recent views. Appa-Rao (1956 *a*) recorded a greater accumulation of total nitrogen in the susceptible type than in the resistant one. Nitrogenous manuring increased the total nitrogen in both types but the suscept still showed a higher N content than the resistant one. Total carbohydrates decreased with nitrogenous manuring in either case, but the susceptible type recorded a higher carbohydrate content than the resistant one indicating, perhaps, its better photosynthetic capabilities. With nitrogenous manuring, the total carbohydrates reached a considerably lower value than in the case of the resistant types. This would again mean that the photosynthates are more efficiently used by the susceptible types to form the necessary α-keto acids *via* the Kreb's TCA cycle to combine with the NH_4 ions and to form the soluble nitrogenous substances like the amides and amino acids. Thus it is conceivable that the enzyme systems related to the assimilation of nitrogen is keyed up better in the suscepts than in the resistant types which is probably intrinsically controlled by a gene or genes and the efficacy of which is largely determined by a critical night temperature round about 20° C. Even though it is not, as yet, clear in what way the resistant types differ from the susceptible types in their pathways of nitrogen utilization, we are inclined to believe from our recent studies that the former seems to have a less pronounced synthetic ability in so far as glutamine is concerned and as judged by its crystallization on the leaf surface. Much emphasis is laid here on glutamine because of its well-known occurrence as a storage reserve in plant parts and the pivotal role it plays in the nitrogen economy of plants and their well-being. It is further appropriate to mention that environmental conditions like long day stimulus and nycto-temperatures, as well as genetic factors, are involved in the biogenesis of glutamine and related compounds in plants (Steward and Pollard, 1956).

It is evident from the above discussion that many factors are involved in determining the degree of resistance/susceptibility of the host to *P. oryzæ*. Apart from the genetical constitution, environment (particularly a low nycto-temperature) exerts a significant influence on host susceptibility. Low nycto-temperatures favour nitrate reduction and consequent synthesis of soluble nitrogenous substances, chiefly amino acids and amides. On the contrary, at high nycto-temperatures, nitrate reduction is possibly low as reported in tomato (Went, 1957) and the products of photosynthesis are largely diverted

into the formation of complex cell-wall material like lignin and cutin which offer resistance to mechanical puncture by the germ tubes. In considering mechanical resistance it is meet to cite the early investigations in Japan bulk of which relates to the deposition of silicon on the epidermal cells providing thereby an outermost barrier to the entry of the germ tubes. A new interpretation on the role of silicon in forming a hypothetical complex with one or more components of the cell-wall, relatively resistant to attack by the extracellular enzymes of *Piricularia*, has been offered recently by Volk *et al.* (1958). Such an assumption has been made plausible in view of the consistent inverse relationship between the silicon content of the rice plant and its susceptibility. Whatever might be the mechanisms underlying these phenomena it is logical to conclude that mechanical resistance is but a reflection of a retarded nitrogen metabolism. An entirely new facet of the problem is the recent emphasis on the function of various organic and inorganic nutrients and nutrilites available on the leaf surface through guttation or by a process of exosmosis and their role in initiating infection. The chemical stimulation of *Piricularia* spores by guttates from rice has been recently reported (Weintraub *et al.*, 1958). These authors found that while a number of sterols, steroidal saprogenins, steroidal amines and α-tocopherol also stimulated germination of spores, these substances could not be identified in the guttates from rice. As has already been pointed out by our investigations, the active fraction in the guttates is mainly glutamine. It is too premature to dwell at length on the importance of cuticular excretions in initiating infection. But by our continued investigations on this question it is becoming increasingly clear that other substances like organic acids and possibly vitamins are also exuded on the rice leaf surface besides glutamine. The situation, however, is analogous to root excretions of which comparatively more is known.

Lastly, our latest finding that maleic hydrazide breaks the resistance of rice to *Piricularia* but only when grown under low nycto-temperatures (Suryanarayanan, unpublished) further strengthens our views on parasitism by the blast fungus. The mechanism of action, however, remains to be investigated, although accumulation of both carbohydrates and glutamine has been reported with other plants treated with maleic hydrazide. As far as we could visualize, the problem of the blast disease of rice should be considered not merely as a case of facultative saprophytism but only at a slightly lower level than obligate parasitism by rusts, etc., because of its exacting growth requirements and its capacity to produce toxins like piricularin and α-picolinic acid (Tamari and Kaji, 1954) and the very many factors that determine host susceptibility at a physiological level. Indeed,

such an understanding of the host-parasite complex would eventually lead to better methods of combating the disease in the field.

I am deeply indebted to my colleague Dr. S. Suryanarayanan, Junior Fellow, National Institute of Sciences of India, for invigorating discussions on the blast problem.

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* Not seen in original.

A SYSTEMATIC ACCOUNT OF THE SOUTH INDIAN CHARACEÆ

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Received October 1, 1957

(Communicated by Prof. M. O. P. Iyengar, F.A.SC.)

THE Indian Characeæ have been the subject of a large number of publications. As many as 25 species, 3 varieties and 5 forms of *Chara*, 31 species, 4 varieties and 2 forms of *Nitella*, 1 species each of *Lychnothamnus* and *Nitellopsis* and 3 species of *Tolypella* are known from the Indian region. Of these only a few have been reported from South India (5 species, and 2 varieties of *Chara* and 1 species and 3 varieties of *Nitella*). A new species of *Nitella* is being described by Prof. Iyengar and will be published elsewhere. The other genera are not yet recorded from South India. The above records from South India are mainly found in the works of older Charophytologists such as Willdenow (1806) and Braun (1849), etc. A few records ar also found in Groves' *Indian Charophyta* (1924).

Thus our present knowledge of the South Indian Characeæ is not in any way comparable to that of the Characeæ of the rest of India.

Prof. M. O. P. Iyengar made a large collection of Characeæ from different localities in South India. He very kindly lent these to the writer for his study. An account of these species is given in this paper.

The Characeæ have been collected from Madras and its neighbourhood, Singaperumalkoil, Vandalur, Guduvancheri, Sriperumbudur, Tiruvellore, Tinnanur, Tirukalikundrum, Mahabalipuram (Seven Pagodas) and Chingleput in the Chingleput District, Tirupati Hills, Renigunta, Mamandur and Nagalapuram in the Chittoor District, Dornakal in the Kurnool District, Ooty in the Ootacamund District, Rameswaram and Pamban in the Ramnad District, Tenmalai in the Travancore State and Bangalore, Nandi Hills, Anantapur and Talguppa in the Mysore State. In all 9 species of *Nitella* and 10 species of *Chara* are recorded in this paper. *Nitella wattii* Groves is reported in this paper and is the second record of this species. A new species of *Chara* is also described, *viz.*, *C. vandalurensis* sp. nov. A key,

* Now at Pachaiyappa's College, Madras-30,

generally adapted from Groves (1924), to the South Indian species is also given in this paper.

Genus **Nitella** Ag.

Key to the South Indian Species of *Nitella*

Branchlets of equal length in a single uniform whorl	HOMŒOCLEMÆ
Dactyls one-celled	ANARTHRODACTYLÆ
Monœcious—Dactyls of sterile whorls upto 500 μ long	*N. acuminata*
Monœcious—Dactyls of sterile whorls much longer	*N. acuminata* var. *belangeri*
Dactyls more than one-celled	ARTHRODACTYLÆ
Dactyls 2-celled	
Diœcious	
Branchlets upto 4 times furcate, dactyls 3–5, unequal	*N. dispersa*
Monœcious	
Dactyls not much abbreviated; ultimate nodes usually fertile	
Tertiary rays 2–3; gametangia produced at all the furcations; Dactyls often of three cells, cells of unequal length ..	*N. mucronata*
Tertiary rays 4–6; Dactyls uniformly 2-celled, cells of uniform size	
Gametangia formed at all the furcations Young furiting whorls not enveloped in mucous cloud, primary rays elongated	*N. pseudoflabellata*
Young fruiting whorls in a mucous cloud; branchlets of unequal length, penultimate rays much abbreviated, 5–6 dactyls	*N. wattii*
Gametangia produced at all furcations except the first	
Plant small, whorls with 6 short and rigid branchlets	*N. tenuissima*

Gametangia produced at all furcations except the ultimate one; Dactyls (some or all) short

Upper cells of coronula not elongate and of the same size as the lower cells

Oogonia solitary dactyls varying in length, not abbreviated .. .. *N. oligospira*

Dactyls abbreviated; Upper cells of coronula elongate and pointed .. .. *N. furcata*

Branchlets of each whorl in more than one series and varying, some longer and compound .. HETEROCLEMÆ

Monœcious, young fertile whorls enveloped in a mucous cloud .. .. .. *N. hyalina*

Nitella acuminata A. Br.

(Text-Figs. 1–7)

Braun, A., 1849, p. 292; Braun & Nordstedt, 1882, p. 35; Groves, J., 1924, p. 365; Zaneveld, 1940, p. 54.

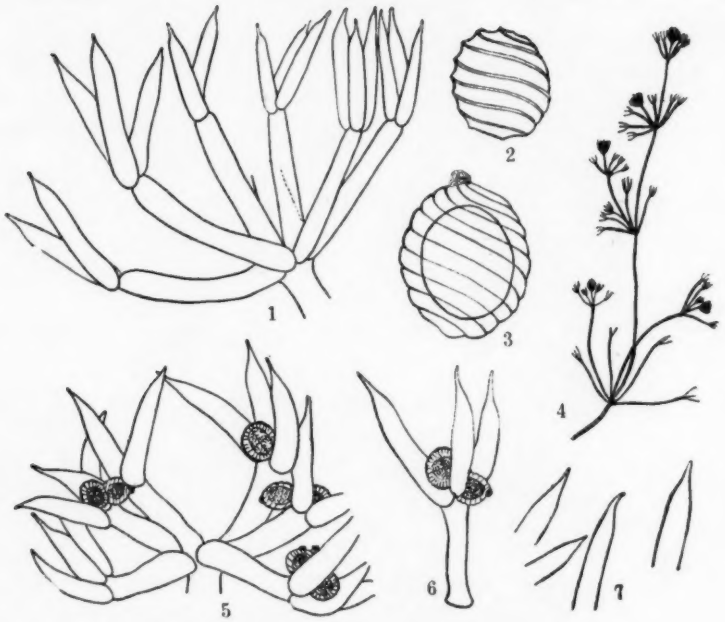
Monœcious. Stem 630–756 μ thick; internodes as long as or longer than the branchlets. Whorls of 6–8 branchlets, once forked. Sterile whorls larger than the fertile whorls; fertile whorls with shorter compact branchlets and forming small heads on special branches. Primary rays more than half the length of the branchlet. Secondary rays 2–3 in sterile whorls, 3–4 in fertile whorls. Dactyls one-celled, apices acuminate. Oogonia and antheridium at furcations of fertile branchlets. Oogonia solitary or geminate or in groups of three, 350–460 μ long, 294–350 μ broad, spiral cells showing about 9 convolutions; coronula small, persistent, 42 μ high, 56 μ broad at the base. Oospores dark brown, 294–308 μ long, 224–238 μ broad, with about 7 ridges. Antheridia solitary, terminal, 252–294 μ in diameter.

Hab.:—In a well near a forest rest house, Tenmalai, Travancore State, October 1922; In a tank, Anantapur, Shimoga District, Mysore State, 22–10–26; In a lake at Ootacamund, Nilgiris; In a pool, at Elliot's Beach, Madras, Jan., 1926, 1928 and 1941 (All leg. M. O .P. I.).

Association: *N. mucronata* (Elliot's Beach Pool); *N. wattii* and *N. furcata* (Anantapur).

The specimens recorded here differ from the type in having narrower stems. In this they resemble the plants collected from Burma (Pal, 1932) and Bengal (Agharkar and Kundu, 1937).

Nitella acuminata A.Br. is differentiated into a number of varieties (*see* Zaneveld, 1940, p. 56). Many of the earlier records of this species do not mention the variety. According to Groves (1922 *a*, p. 98) the length of the primary rays and dactyls and the presence of solitary or geminate oogonia are very variable and as such cannot be relied upon to serve as grounds for the separation of the varieties (*cf.* Zaneveld, 1940, pp. 51 and 59).



TEXT-FIGS. 1–7. *Nitella acuminata.*

Fig. 1. Sterile whorl of branchlets. Fig. 2. Oospore. Fig. 3. Oogonium. Fig. 4. Habit. Fig. 5. Fertile whorl of branchlets. Fig. 6. A fertile branchlet. Fig. 7. Apices of dactyls. (Fig. 1, × 10. Figs. 2, 3, × 54. Fig. 4, × ⅓. Figs. 5, 7, × 17. Fig. 6, × 22.)

The writer's observations on the different South Indian collections of *N. acuminata* confirm this view. The length of the dactyls was very variable in the different specimens. Hence he is unable to definitely place the different plants in one or the other variety.

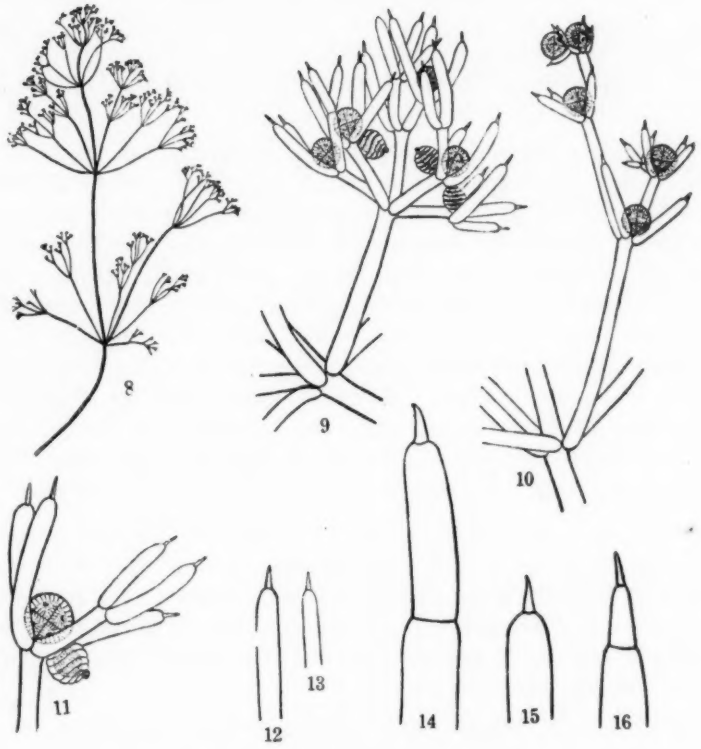
Nitella dispersa A.Br.

(Text-Figs. 8, 10, 12, 13)

Braun and Nordstedt, 1882, p. 47; Groves, J., 1924, p. 365.

Diœcious. Stem 574–602 μ thick. Whorls of 6–7 branchlets, about 15 mm. long; upto 4 times furcate. Primary rays half as long as the entire

branchlet or longer. Rays at first furcation usually 4, of which two are simple, others furcate; rays at second furcation 3–4 of which one is again furcate; rays at third furcation 3–4 of which one is again furcate; rays at fourth furcation 3. Dactyls unequal, uniformly two-celled, lower cell more or less rounded at the apex, upper cell conical. Antheridia in all the furcations, 420–448 μ in diameter. Female plants not seen.



TEXT-FIGS. 8–16. *Nitella dispersa & N. mucronata.*

Figs. 8, 10, 12, 13. *N. dispersa.* Fig. 8. Habit. Fig. 10. A single branchlet. Figs. 12, 13. Apices of dactyls. (Fig. 8, × ¾. Fig. 10, × 10. Figs. 12, 13, × 22.)

Figs. 9, 11, 14–16. *N. mucronata.* Fig. 9. A single branchlet. Fig. 11. A single node of branchlet. Figs. 14, 16. Apices of 3-celled dactyls. Fig. 15. Apex of 2-celled dactyl. (Fig. 9, × 17. Fig. 11, × 30. Figs. 14–16, × 86.)

Hab.: In a pond at Mamandur, Chittoor District, 18-2-30 (Leg. M. O. P. I.).

Association: *Nitella mucronata* and *N. oligospira.*

This species is recorded for the first time from South India. It is very peculiar in having antheridia at all nodes including the first and the ultimate nodes. Agharkar and Kundu (1937, p. 5) report that the gametangia are found arranged in all the nodes except the first node.

Nitella mucronata (A.Br.) Miquel

(Text-Figs. 9, 11, 14–16)

Braun and Nordstedt, 1882, p. 50; Migula, 1897, p. 149, figs. 42–44; Groves, J. and Bullock-Webster, 1920, p. 113, pls. 1 & 12; Groves, J., 1924, p. 366; Zaneveld, 1940, p. 95.

Monœcious. Stem 392–490 μ thick; internodes 1–3 times the length of the branchlets, whorls of 5–6 branchlets; sterile and fertile branchlets similar. Branchlets 2–3 times furcate. Primary rays about half the length of the entire branchlet or slightly less. Rays at the first furcation 3–5; at the second 2–3, rarely 4, of which one is again furcate; at the third furcation 2–3. Dactyls 2-, rarely 3-celled, lower cell generally rounded at the apex, occasionally tapering at the apex; ultimate cell narrow, conical, acute and mucro-like. Gametangia usually produced at all the furcations of the branchlet. Oogonia solitary, 392–462 μ long, 336–378 μ broad; spiral cells showing 8–9 convolutions, coronula 42 μ high, 70 μ broad at the base. Oospores 280–308 μ long, 238–252 μ broad with the outer membrane translucent and reticulate. Antheridia solitary, 266–280 μ in diameter.

Hab.: In an artificial tank in a Fern House, in the A.H. Gardens, Madras, 2–8–23; in a swamp, Malleswaram, Bangalore, in Mysore State, 26–6–27; in a ditch by the side of the Railway cutting, Tenmalai, in Travancore State, 4–10–24; in a pool, Dornakal, 31–12–30; in a pool at Elliot's Beach, Madras, 20–1–25 and January 1928; in a pool at Mamandur near Renigunta Jn., 18–3–30 (All leg. M. O. P. I.).

Association: *N. dispersa* and *N. oligospira* (Mamandur), *N. acuminata* (Elliot's Beach) and *N. oligospira* (A. H. Gardens).

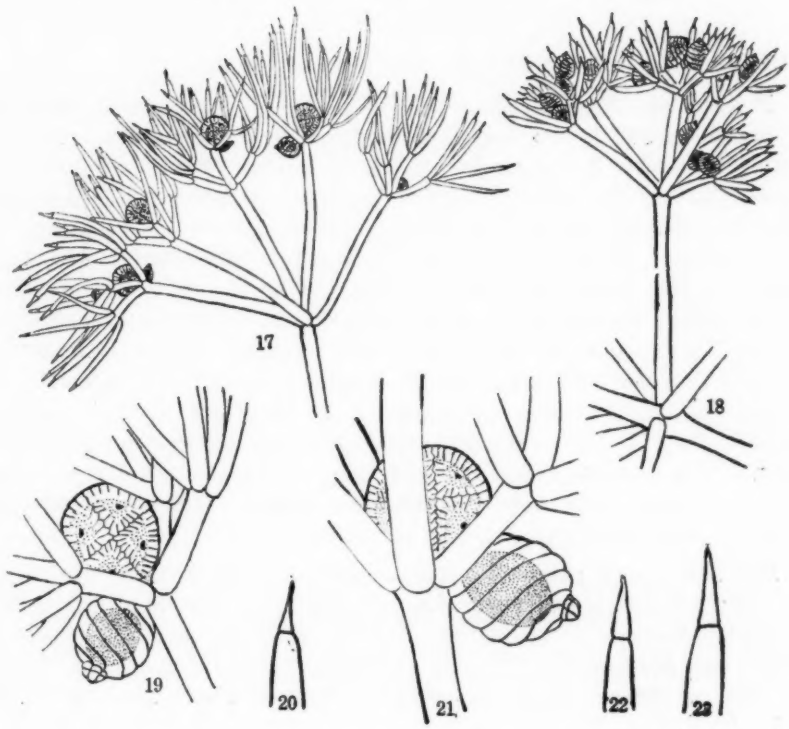
Nitella pseudoflabellata A.Br.

(Text-Figs. 18, 20, 21)

Braun and Nordstedt, 1882, p. 54; Groves, J., 1924, p. 366; Zaneveld, 1940, p. 73, figs. 7 *a–e*.

Monœcious. Stem 224–238 μ thick; internodes 1–1½ times as long as the branchlets. Whorls of 5–8 branchlets, somewhat spreading and stiff

with bunched tips. Branchlets 2–3 times furcate. Primary rays about 3/5 the length of the entire branchlet; rays at the first furcation 6–7; at the second furcation 5–6 of which often 2–3 are again furcate. Dactyls 4–5, straight, 2-celled; lower cell acuminate truncate at the apex, ultimate cell deciduous, narrow, conical with a pointed tip which is often bent, 21–28 μ broad at the base, 70–98 μ long. Gametangia produced at the second and third furcations of the branchlet. Oogonia solitary ellipsoid, 280–308 μ long, 224–252 μ broad with 8–9 convolutions; coronula 28–35 μ high, 42–49 μ broad at the base. Oospores 266–294 μ long, 238–266 μ broad; oospore membrane reticulate. Antheridia 182–196 μ in diameter.



TEXT-FIGS. 17–23. *Nitella pseudoflabellata & N. tenuissima.*

Figs. 18, 20, 21. *N. pseudoflabellata.* Fig. 18. A branchlet. Fig. 20. Apex of dactyl. Fig. 21. A fertile node of a branchlet. (Fig. 18, × 17. Figs. 20, 21, × 86).

Figs. 17, 19, 22–23. *N. tenuissima.* Fig. 17. Tip of branchlet. Fig. 19. Branchlet node with gametangia. Figs. 22, 23. Apices of dactyls. (Fig. 17, × 30. Fig. 19, × 86. Figs. 22, 23, × 150).

Hab.: In a pool in Elliot's Beach, Madras, February 1925 (Leg. M. O. P. I.)

This species includes two varieties, var. *mucosa* (Nordst.) Bailey and var. *mutila* A.Br. (see Zaneveld, 1940, p. 75). But the varietal identification can be done only when one works with a fresh collection of the specimen. As the present study is based on preserved material, the author is unable to differentiate the present form into one or the other variety. The present alga, however, is very narrow and has smaller antheridia and oogonia.

Nitella wattii Groves, J.

(Text-Figs. 24–29)

Groves, J., 1924, p. 367, pl. 36; Zaneveld, 1940, p. 82.

Monœcious. Stem slender; 200–560 μ thick; internodes as long as the branchlets or slightly longer. Whorls of 6–7 branchlets of unequal length. Branchlets 2–3 times furcate, rarely 4 times furcate. Primary ray exceeding half of the length of entire branchlet; secondary rays 5–7 usually elongated; tertiary rays 4–7, some simple, some forked, the latter usually very short, *i.e.*, the penultimate cells much abbreviated; dactyls 4–6, very slender, 35–42 μ broad, uniformly 2-celled, the lower cell much curved at the base slightly tapering at the apex; ultimate cell elongate-conical, 49–56 μ high, 17–21 μ broad at the base, with a long acuminate point. Gametangia enveloped in mucus. Oogonia solitary usually produced at the second and the third nodes, antheridia sometimes in all the three nodes. Oogonia 238–280 μ long, 168–224 μ broad, spiral cells showing 8–9 convolutions; coronula 28 μ high, 42 μ broad at the base, oospore 210–238 μ long, 182–224 μ broad with 7–8 inconspicuous ridges; oospore membrane reticulate. Antheridia 210–238 μ in diameter.

Hab.: In a small pool, at Talguppa in Shimoga District, Mysore State, 24–11–26; in a tank at Anantapur, Shimoga District, Mysore State, 20–10–26; in a pool at Vandalur, near Madras, 17–1–41; 27–3–42; in a tank at Guduvancheri near Madras, 17–3–23; in paddy-fields, at Adyar, Madras, 24–12–40; in a tank, at Nagalapuram near Nagari, Chittor Dist., Jan. 1933 (All leg. M. O. P. I.).

Association: *N. hyalina* and *C. flaccida* (Guduvancheri); *N. furcata* (Adyar paddy-fields); *N. furcata* and *N. acuminata* (Anantapur).

This species was first described by J. Groves (1924) from a specimen collected from Mugra by Sir George Watt (No. 306). It has not so far been recorded from any other place either from India or from abroad.

The present report of this species from diverse localities in South India is the second record of this species.

This species is distinguished by " the unequal length of branchlets in the same whorl and the much abbreviated penultimate rays, surmounted by the cluster of dactyls, presenting a tassel-like appearance". This is so in all the South Indian collections of *N. wattii*.

TEXT-FIGS. 24–29. *Nitella wattii*.

Fig. 24. Branchlet of a form from Vandalur. Fig. 25. A branchlet node with oogonium. Fig. 26. Apex of dactyl. Fig. 27. Branchlet of a form from Talguppa. Fig. 28. A dactyl. Fig. 29. Habit. (Fig. 24, × 10. Fig. 25, × 150. Fig. 26, × 217. Fig. 27, × 17. Fig. 28, × 53. Fig. 29, × ½.)

Groves (1924, p. 367) mentions " The mucus cloud is hardly perceptible to the naked eye in the dried plant". The writer collected fresh material of this species from the paddy-fields at Adyar and in this he observed that the fruiting whorls were enveloped in a mucus cloud and presented a pretty appearance.

The arrangement of the gametangia in this species is very interesting (Groves, 1924, p. 367, *cf.* Zaneveld, 1940, p. 82). According to Groves the antheridia are usually produced at the second and sometimes at the first node, while the oogonia are produced at the 3rd and 4th and occasionally at the second. The writer's observations on the South Indian material go to show that this is a very variable character. Even in one and the same collection this character was varying from plant to plant. In the Vandalur and the Adyar forms the gametangia were arranged in the manner described by Groves. In the Talguppa material, for instance, the antheridia were mostly found at the first furcation, though they were also found at the second furcation. Similarly the oogonia were found at the second and the third furcations. Plants collected from Anantapur similarly showed gametangia at all the furcations.

Nitella tenuissima (Desv.) KÜTZING

(Text-Figs. 17, 19, 22, 23)

Braun and Nordstedt, 1882, p. 62; Migula, 1897, p. 173, figs. 48–49; Groves, J., and Bullock-Webster, G. R., 1920, p. 120, pl. 14.

Monœcious, small, 2–3 inches in height; stem slender, 154–168 μ in thickness, internodes about twice as long as the branchlets. Whorls usually of 6 short, somewhat spreading branchlets. Branchlets 3–4 times furcate. Primary rays about 2/3 the entire length of the branchlet; secondary rays 4–6, tertiary rays 4–6, quaternary rays 4–5 of which one is, occasionally, again furcate with 3–4 quinary rays. Dactyls long, narrow and straight, 2-celled; lower cell of uniform thickness, usually truncate at the apex and a little broader than the base of the ultimate cell, ultimate cell long, narrow and acuminate. Gametangia formed at the second and the third furcations and absent at the first furcation, often both oogonia and antheridia together. Oogonia solitary, 224–252 μ long and 182–210 μ broad, coronula 25 μ high, and 42 μ broad at the base, persistent, spiral cells showing about 7 convolutions. Antheridia 154–182 μ in diameter. Oospore not seen.

Hab.: In a pool at Vandalur, near Madras, 7–12–40 (Leg. M. O. P. I.). This alga resembles very closely the type.

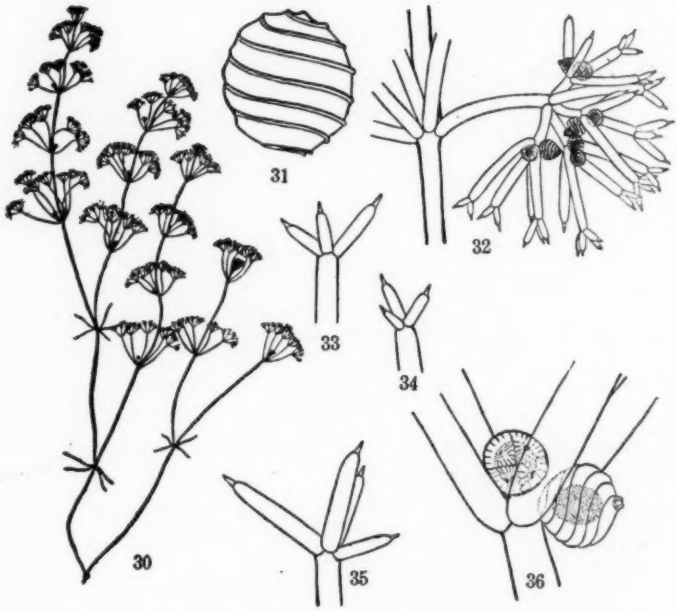
N. tenuissima has been recorded from India only twice, first by Braun (1849, p. 294) and later by Dixit (1940, p. 232). Both the plants recorded by these two authors belong to var. *byssoides* A.Br. The present record is of interest in that it completely agrees with the original description of the species. It differs from *N. tenuissima* var. *byssoides* in the much branched habit and the thickness of the stem (*see* Dixit, 1940, p. 232).

Nitella oligospira BRAUN

(Text-Figs. 30–36)

Braun and Nordstedt, 1882, p. 67, pl. 2, figs. 50–52; Groves, J., 1924, p. 368; Zaneveld, 1940, p. 82.

Monœcious. Stem slender 364–490 μ thick; internodes 1–2 times the length of the branchlets. Whorls of 5–7 straight or slightly spreading branchlets. Branchlets 2–3 times furcate. Primary rays about half the length of the entire branchlet; secondary rays 4–6; tertiary rays 3–5 of which 1–2 are simple, others furcate into 2–4 quaternary rays. Dactyls variable in length, some very long, some much abbreviated, 2-celled, lower cell tapering



TEXT-FIGS. 30–36. *Nitella oligospira.*

Fig. 30. Habit. Fig. 31. Oospore. Fig. 32. A branchlet. Figs. 33–35. Ultimate furcations showing the dactyls. Fig. 36. Branchlet with antheridium and oogonium (Fig. 30, × ⅓. Fig. 31, × 54. Fig. 32, × 10. Figs. 33–35, × 22. Fig. 36, × 36.)

or rounded or truncate at apex, which is little broader than the base of the ultimate cell; ultimate cell narrow-conical, 35 μ broad at base, 84 μ long. Oogonia and antheridia usually found at the second and third furcations, rarely on the first also. Oogonia solitary, 350–448 μ long, 294–350 μ broad,

spiral cells showing 7–9 convolutions, coronula 56 μ high, 70 μ broad at the base. Oospores 364–392 μ long, 350–364 μ broad, showing about 7 ridges; oospore membrane reticulate. Antheridia 238–252 μ in diameter.

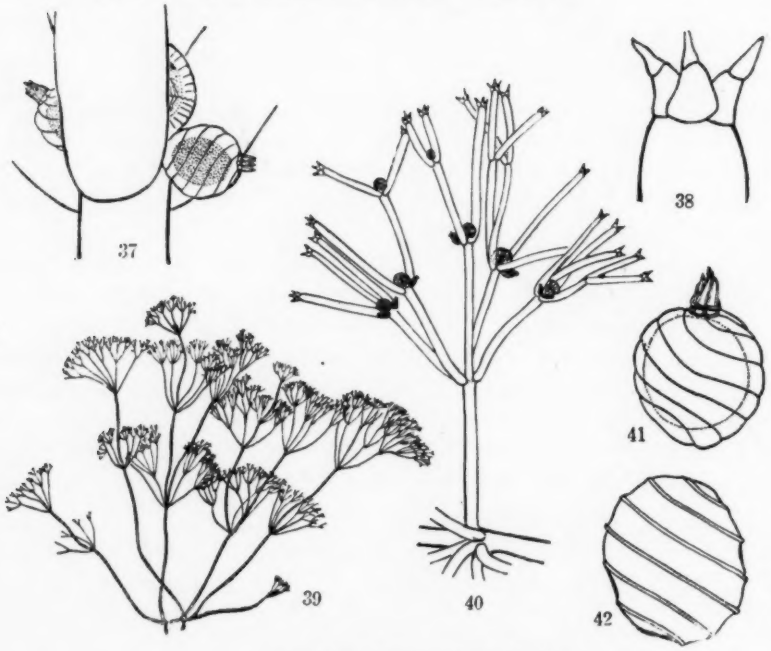
Hab.: In a pool in Mamandur, near Renigunta, Chittoor Dist., 18–3–30; in an artificial tank in the A. H. Gardens, Teynampet, Madras, 2–8–23; in a pool at Elliot's Beach, Madras, 24–1–25; in a tank in Talguppa, in Shimoga District, Mysore State, 24–10–26; in a pool at Vandalur, near Madras, February 1923; in a pool at Seven Pagodas (All leg. M. O. P. I.).

Association: *N. mucronata*, *N. dispersa* (Mamandur); *C. brachypus*, *C. braunii* (Seven Pagodas); *C. erythrogyna* (Talguppa); *N. mucronata* (A.H. Gardens, Madras).

Nitella furcata (Roxb.) Agardh

(Text-Figs. 37–42)

Groves, J., 1924, p. 368; Zaneveld, 1940, p. 88.



TEXT-FIGS. 37–42. *Nitella furcata*.

Fig. 37. Branchlet node showing the antheridium and the two oogonia. Fig. 38. Ultimate furcation of a branchlet showing the abbreviated dactyls. Fig. 39. Habit. Fig. 40. A branchlet. Fig. 41. Oogonium showing the upper coronal cells elongated to a point. Fig. 42. Oospore. (Fig. 37, × 54. Figs. 38, 41, 42, × 86. Fig. 39, × $\frac{1}{3}$. Fig. 40, × 10.)

Monœcious. Stem stout, 588–672 μ thick; internodes 1–2 times the length of the branchlets. Whorls of 5–6 spreading branchlets; 3–4 times furcate; Primary rays exceeding half the length of the entire branchlet or slightly less; secondary rays 4–6, tertiary rays usually 2–3 (4); quaternary rays 2–3. Dactyls abbreviated, divergent, uniformly 2-celled, lower cell tapering but truncate at apex, which is of the same breadth as the base of the terminal cell. Ultimate cell conical or slightly acuminate, 70 μ long, 42 μ broad at the base. Gametangia usually borne at all furcations except the ultimate one. Oogonia clustered, 350–518 μ long, 294–392 μ broad, spiral cells showing 8–9 convolutions, coronula 84 μ high, 56 μ broad at base, cells of upper tier of coronula elongated to fine points. Oospore 308 μ long, 238 μ broad, showing about 6 ridges, oospore membrane reticulate. Antheridium solitary, 294–322 μ in diameter.

Hab.: In a pond in the Madras Museum compound in Egmore, Madras, 3-4-42; in paddy-fields in Adyar, near Madras, 24-12-40; in a tank at Vandalur, near Madras, Feb. 1923 and 16-1-25; in a tank in Anantapur, Shimoga District, Mysore State, 22-10-26 (All leg M. O. P. I.).

Association: *N. wattii* (Adyar, Madras); *N. wattii* and *N. acuminata* (Anantapur).

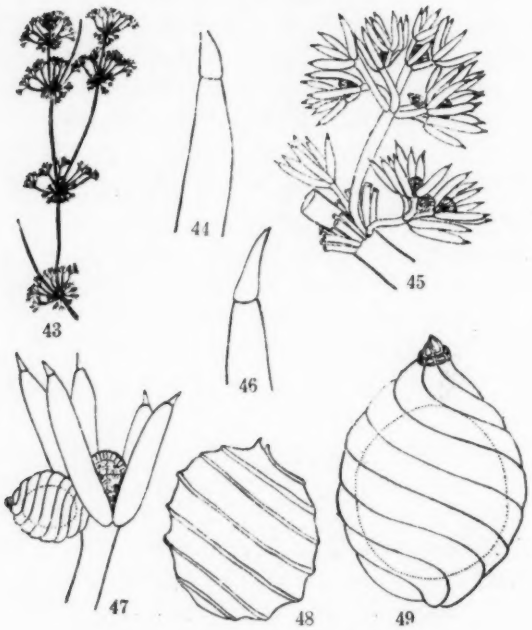
Nitella hyalina (DC) Agardh

(Text-Figs. 43–49)

Braun and Nordstedt, 1882, p. 78; Migula, 1897, p. 190, figs. 55–57; Groves, J. and Bullock-Webster, G. R., 1920, p. 127, pl. 5, fig. 7 and pl. 16; Groves, J., 1924, p. 369; Zaneveld, 1940, p. 108.

Monœcious; younger whorls enveloped in mucilage; Stem 336–476 μ thick; internodes 2–4 times the length of the branchlets. Whorls of usually 6–7 primary branchlets. Accessory branchlets double the number of primary branchlets in two series, one above and the other below the primary branchlets. Primary branchlets 2–3 times furcate, primary rays $\frac{1}{2}$–$\frac{2}{3}$ the length of the entire branchlet; secondary rays 6–7; tertiary rays 4–7, sometimes again furcate into 4–6 quaternary rays. Ultimate rays uniformly 2-celled, the lower cell narrowing gradually to the base of the ultimate cell; ultimate cell narrowly conical, acute, 84 μ long, 35 μ broad at the base. Accessory branchlets usually one above and one below each primary branchlet; those of the upper series once furcate into 5–6 rays, those of the lower series 1–2 times furcate; rays at the first furcation 5–6 of which 2–3 are simple and the others furcate into 4–5 tertiary rays. Gametangia enveloped in mucus; usually produced on the primary branchlets at the

second, third, more rarely at the first, furcations and some times also on the accessory branchlets. Oogonia solitary, 364–462 μ long, 308–350 μ broad; spiral cells showing about 9 convolutions; coronula 42–56 μ high, 56–63 μ broad at the base. Oospore dark brown, 294–350 μ long, 238–280 μ broad with 7 ridges. Antheridia 252–280 μ in diameter.



TEXT-FIGS. 43–49. *Nitella hyalina.*

Fig. 43. Habit. Figs. 44, 46. Apices of dactyls. Fig. 45. A primary branchlet and two secondary branchlets. Fig. 47. Branchlet node with antheridium and oogonium. Fig. 48. Oospore. Fig. 49. Oogonium. (Fig. 43, × $\frac{3}{4}$. Figs. 44, 46, 48, 49, × 86. Fig. 45, × 10. Fig. 47, × 30.)

Hab.: In a marsh at Ennore, near Madras, 7-3-40; in a lake at Sriperumbudur, near Madras, 23-2-35; in a pool at Vandalur, near Madras, 10-3-42; in a lake at Chingleput, near Madras, 27-3-42; Godavari, 1908; in the tank at Red Hills, near Madras, 31-8-29; in a tank at Guduvancheri near Madras, 17-3-23 (All leg. M. O. P. I.).

Association: *C. flaccida* and *C. zeylanica* (Ennore and Red Hills); *N. wattii* (Guduvancheri).

Genus **Chara** Linn.

Key to the South Indian species of *Chara*.

Stipulodes in a single whorl	HAPLOSTEPHANÆ
Entirely ecorticate	
Gametangia produced at the base of the whorl and at the branchlet nodes, stipulodes rudimentary, monœcious	*C. corallina*
Gametangia not produced at the base of the whorl	
Stipulodes more or less well developed; Monœcious; Gametangia aggregated, branchlets with Corona-like apices ..	*C. braunii*
Stipulodes rudimentary, Gametangia solitary, branchlets without Corona-like apices ..	*C. nuda*
Stem corticate	
Branchlets ecorticate	
Oogonia and antheridia produced at different branchlet nodes	*C. erythrogyna*
Oogonia and antheridia produced at the same branchlet nodes	
Ripe oospores brownish	*C. flaccida*
Ripe oospores black	*C. gymnopitys*
Branchlet segments, except the first and one or two ultimate, corticate	*C. hydropitys*
Stipulodes in a double circle	DIPLOSTEPHANÆ
Rows of cortical cells of the stem thrice the number of branchlets	Triplostichæ
Branchlets ecorticate	
Stipulodes well developed, lowest segment long; spine-cells well developed ..	*C. handæ**
Lower row of Stipulodes rudimentary; bracteoles shorter than the oogonium, lowest segment short; spine cells rudimentary ..	*C. vandalurensis*

* Not recorded from South India.

Branchlet segments corticate

Lowest branchlet segment short, corticate; stipulodes elongated, cortical cells of branchlets thrice as many as the bract cells; spine cells minute, acute .. *C. brachypus*

Lowest branchlet segment ecorticate, cortical cells of branchlets thrice as many as the bract cells; stipulodes elongated; spine cells acute .. .. .. *C. zeylanica*

Chara corallina Willd.

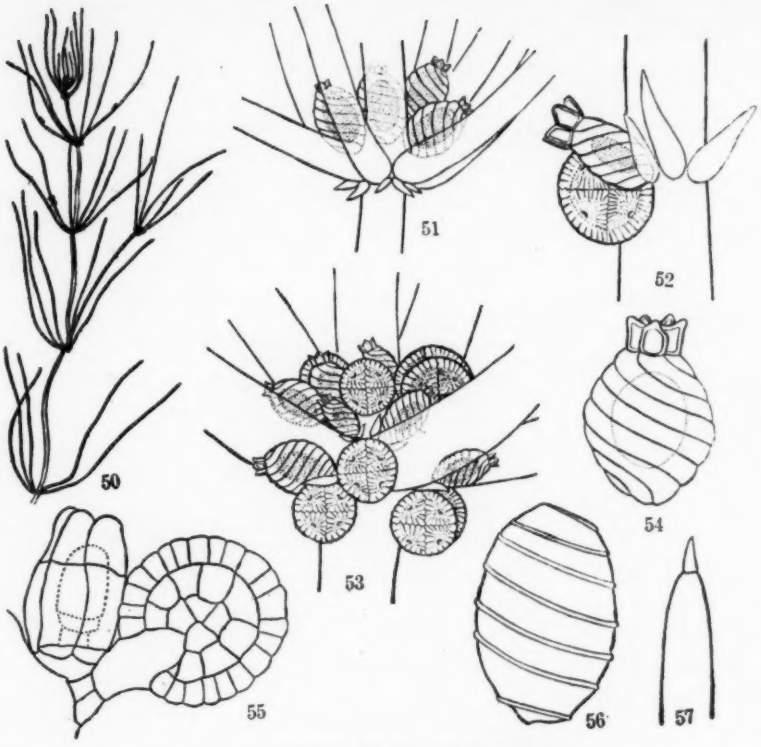
(Text-Figs. 50–57)

Willdenow, C. L., 1806, p. 61, pl. 22, fig. 2.; Braun, A., 1849, p. 294; Braun and Nordstedt, 1882, p. 108, pl. 7, fig. 199; Groves, J., 1924, p. 371; Zaneveld, 1940, p. 131, fig. 12 *a*.

Monœcious. Stem stout, 770–840 μ thick; internodes long, but often exceeded in length by the branchlets. Stem and branchlets entirely ecorticate. Stipulodes usually rudimentary, single row, short and acute, alternating with the branchlets. Whorls of 5–7 branchlets. Branchlets of about 4 swollen segments, the ultimate one being short and acute. Bract-cells 3–4, small, acute, at all branchlet nodes, sometimes absent on the ultimate node. Gametangia produced at the base of the whorls as well as at the lower branchlet nodes. Antheridia and oognia appear to grow side by side when occurring on a free branchlet node. Oogonia large, 840–1008 μ long, 532–756 μ broad, spiral cells showing 8–9 convolutions; coronula 126–154 μ high and 182–196 μ broad at the base. Oospore black, 742–840 μ long, 490–560 μ broad, showing about 7 ridges. Antheridia 504–588 μ in diameter.

Hab.: In a small tank inside a Fern House in the Agri-horticultural Gardens, Madras, 14-9-24; in a pool at Mandasa, Vizag District, 2-1-36; in a pool near Elliot's Beach, Madras; in a pond in the Madras Museum, 15-5-57 (All leg. M. O. P. I.).

This species is characterised by the arrangement of the gametangia in a diagonal position in relation to the node and which therefore appear to grow side by side. But when the very early stages of the gametangia are examined, the origin of the antheridium and the oogonium from the same peripheral cell could be made out very clearly (Fig. 55; *see also* Agharkar and Kundu, 1937, p. 12).



TEXT-FIGS. 50–57. *Chara corallina.*

Fig. 50. Habit. Fig. 51. Stem node showing stipulodes and also ripe oogonia. Fig. 52. Node of branchlet with gametangia. Fig. 53. Gametangia at the base of the whorl of branchlets. Fig. 54. Oogonium. Fig. 55. Very early stage of the antheridium and the oogonium showing their origin from the same peripheral cell. Fig. 56. Oospore. Fig. 57. Apex of branchlet. (Fig. 50, × ½. Fig. 51, × 10. Figs. 52, 54, 56, × 36. Fig. 53, × 17. Fig. 55, × 150. Fig. 57, × 22.)

Chara braunii Gmelin

(Text-Figs. 58–64)

Groves, J., and Bullock-Webster, 1924, p. 11, pl. 26; Groves, J., 1924, p. 372; Zaneveld, 1940, p. 137.

=*Chara coronata* Ziz., Braun, A., 1849, p. 295; Braun and Nordstedt, 1882, p. 108; Migula, 1897, p. 321, figs. 81–83; Migula, 1925, p. 225, fig. VII, 1, 2.

Monœcious. Stem moderately stout, 420–700 μ thick, internodes usually about the same length as branchlets or a little longer. Stem and

branchlets entirely ecorticate. Whorls of 8–9 branchlets. Stipulodes single row, well developed, as many as the branchlets and alternating with them, spreading, variable in size, acute. Branchlets usually straight or slightly incurved, of 4–5 segments, the lower 3–4 elongated, the uppermost segment very short and scarcely recognizable, forming along with the bracts of the uppermost node, a corona-like apex, acuminate. Bract-cells usually 3. Bracteoles equal to or exceeding the fruit, 1½–2 times in length. Oogonia and antheridia produced at the two lowermost branchlet nodes, solitary, occasionally geminate, absent at the base of the branchlet whorls. Oogonium 560–700 μ long, 336–448 μ broad; spiral cells showing about 8–10 convolutions; coronula 140–182 μ high, 182–238 μ broad at the base, ovoid conical with rounded apex. Oospore black, ellipsoid-cylindrical, 434–560 μ long, 294–392 μ broad, showing about 8–9 inconspicuous ridges terminating in short basal claws; oospore membrane finely granular. Antheridium 280–350 μ in diameter.

Hab.: In a swamp at Malleswaram in Bangalore, Mysore State, 28-6-27; in a pool, at Seven Pagodas, Madras; Nandi Hills, Mysore State (All leg. **M. O. P. I.**).

The plants were not incrusted and were about 10 cm. high. In general, the gametangia are smaller than in the type. In the Malleswaram form the stem internodes were longer than the branchlets and the fruits were smaller than those of the Seven Pagodas form.

So far there has been one record of this species from the Madras State, *viz.*, *C. braunii* var. *coromandelina*.

A large number of varieties are known in the species. Zaneveld (1940, p. 139) examined type materials of the different varieties and on the basis of his study he distinguished as many as six varieties. These varieties are var. *braunii*, var. *schweinitzii*, var. *coromandelina*, var. *perrottetii* and var. *oahuensis*. To these he adds a new variety, var. *kurzii* Zanev., based on a study of Kurz's original material which Braun described as var. *coromandelina*. He differentiates these varieties on the basis of the length of the oospores, the number of ridges, the length of the bract-cells in relation to the length of the oogonium, situation of the bract cells and the shape of the coronula cells. Using these characteristics as the basis the writer tried to identify the different specimens of *C. braunii*. The specimens collected from Seven Pagodas seem to closely agree with the var. *coromandelina*. The description of the variety is as follows:

Var. *coromandelina* (A.Br.) Zaneveld. The Charophyta of Malaysia, 1940, p. 144.

= *Chara coronata* Ziz, var. *coromandelina* Br., in Braun and Nordstedt, 1882, *pro parte*.

Plants moderately robust. Stem 420–476 μ thick, internodes as long as the branchlets. Branchlets 8–9, straight or sometimes incurved, with 4–5 segments, end segment very short often not recognizable from the bracts of the last segment. Stipulodes in a single row, well developed, as many as the branchlets and alternating with them. Bract-cells 3, verticillate, anterior ones as long as the oogonium and the posterior ones short, acuminate, 402–700 μ in length. Bracteoles variable in length, equal to or a little longer than the oogonium. Gametangia formed at the two lowermost branchlet nodes, usually solitary or rarely geminate. Oogonia 616–700 μ long and 336–448 μ broad, with 8–9, rarely 10, convolutions; coronula 154–182 μ high and 210–238 μ broad at the base, with ovoid-conical cells with rounded apices. Oospores black, ellipsoid-cylindrical, 504–560 μ long and 364–392 μ broad with 8–9 inconspicuous ridges. Antheridia 308–350 μ in diameter.

Hab.: In a pool at Seven Pagodas, Madras State.

One another variety of *C. braunii* is the one collected from Malleswaram. This plant differs much from the Seven Pagodas form. The Malleswaram form agrees with var. *kurzii* in the bracts and the bracteoles being up to double as long as the oogonia, and in the absence of difference in the development of the anterior and the posterior bracts and bracteoles and in the number of ridges. However, it may be pointed out here that it differs in the smaller oospore-dimensions, and hence is kept here as a separate form.

Var. *kurzii* Zanev. Charophyta of Malaysia, 1940, p. 144.

= *Chara coronata* Ziz. var. *coromandelina* A.Br. in Braun and Nordstedt, *Fragmente einer monographie der Characeen*, 1882, 112, *pro parte*.

forma.

Plant brittle. Stem 658–700 μ thick, internodes longer than the branchlets. Branchlets 9, slightly incurved, with 5 segments, end segment scarcely recognisable along with the bracts of the topmost node. Stipulodes single row, well-developed spreading acuminate. Bract-cells three. Bracteoles longer than the fruit, 1½ times sometimes upto twice as long as the fruit. Gametangia produced at the two lowermost branchlet nodes usually solitary. Oogonia 560–588 μ long and 392–420 μ broad with about 9 convolutions; coronula 140–154 μ high and 182–210 μ broad at base; oospores black

TEXT-FIGS. 58–70. *Chara braunii* & *C. nuda*.

Figs. 58–64. *C. braunii*. Fig. 58. Branchlet node with two pairs of antheridia and oogonia. Fig. 59. Terminal segment of branchlet. Fig. 60. Branchlet node with a single antheridium and a single oogonium. Fig. 61. Habit. Fig. 62. Stem node showing the stipulodes. Fig. 63. A whole branchlet. Fig. 64. Oospore. (Fig. 58, × 22. Fig. 59, 60, 64, × 36. Fig. 61, × ⅓. Fig. 62, × 17. Fig. 63, × 10.)

Figs. 65–70. *C. nuda* Fig. 65. Habit. Fig. 66. Stem node showing stipulodes. Fig. 67. Oospore. Fig. 68. Branchlet node with gametangia. Fig. 69. Apex of branchlet. Fig. 70. A whole branchlet. (Fig. 65, natural size. Fig. 66, × 17. Fig. 67, × 54. Fig. 68, × 36. Fig. 69, × 30. Fig. 70, × 10.)

ellipsoid-cylindrical, 434–476 μ long, 294–322 μ broad with 8–9 inconspicuous ridges terminating in short basal claws. Antheridia 280–294 μ in diameter.

Hab.: In a swamp at Malleswaram in Bangalore, Mysore State.

Chara nuda Pal

(Text-Figs. 65–70)

Pal, B. P., 1932, p. 81, pl. 15; Zaneveld, 1940, p. 152.

forma.

Monœcious. Stem and branchlets entirely ecorticate. Stem 434–476 μ thick. Internodes shorter than the branchlets, sometimes less than half as long. Stipulodes in a single circle, usually rudimentary and irregular, acute. Whorls with 8–9 branchlets, which are incurved, lowermost segment short, terminal cell narrow and short, tapering to an acute point. Bracts rudimentary. Bracteoles acute, about half as long as the fruit. Gametangia usually at the two lowest nodes, absent at the base of the whorls. Oogonia solitary, 672–742 μ long, 504–518 μ broad; spiral cells showing about 10–12 convolutions; coronula 126–140 μ high, 224–238 μ broad at the base, short flattened with sometimes a slight constriction between it and the apices of the spiral cells. Oospore oval-ellipsoid, dark brown, 518–560 μ long, 364–392 μ broad, showing 10–12 ridges ending in short basal claws. Antheridium small, 294 μ in diameter.

Hab.: In a paddy-field drain, Singaperumal Koil, near Madras, 9-1-29 (Leg. M. O. P. I.).

Association: *C. hydropitys.*

This plant which reaches only about 2–3 inches in height is incrusted with lime.

There are two species known among the ecorticate haplostephanous monœcious species of *Chara* which have rudimentary stipulodes, *C. nuda* Pal and *C. pashanii* Dixit. These are essentially characterised by the absence of gametangia at the base of the whorls. Though Dixit (1935, p. 258) mentions the absence of stipulodes as a distinguishing feature, Zaneveld (1940, p. 151) on a study of Dixit's material states that he found rudimentary stipulodes in *C. pashanii* Dixit. The present plant agrees very closely with *C. nuda* Pal.

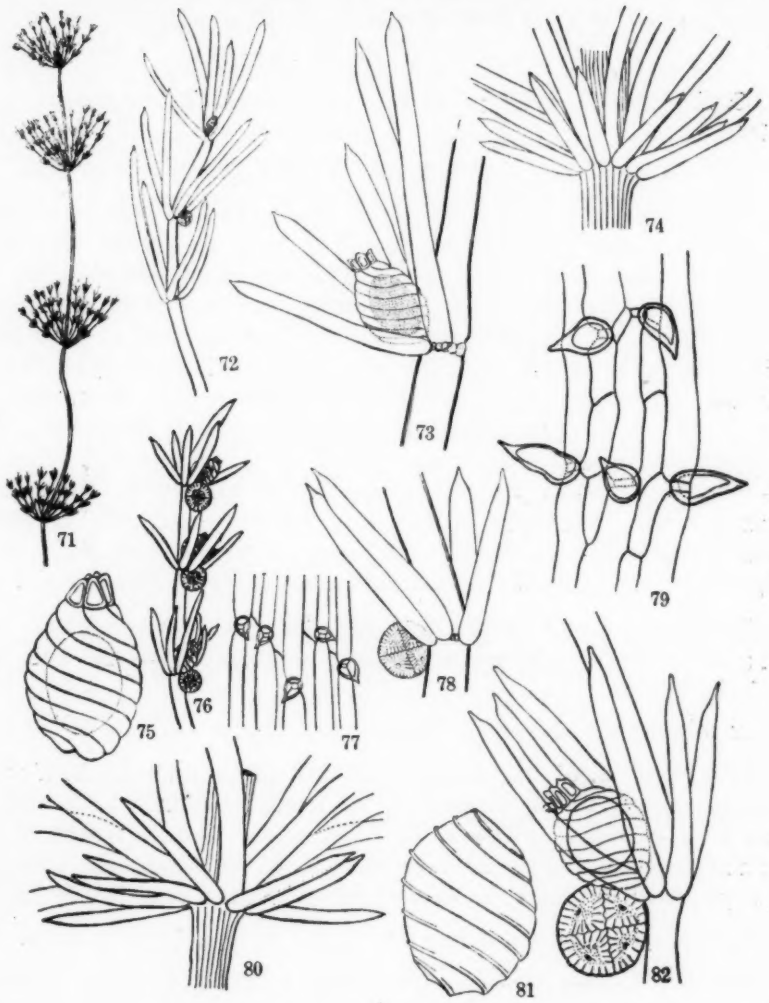
Dixit (1940, p. 234) described a new form of this species, f. *kolhapurensis* Dixit, which differs from the type in the branchlets being 11 in a whorl in the dimensions of the gametangia, and also in the rudimentary bracteoles,

Table I gives a comparative statement of the dimensions of the Burmese, the Bombay and the Madras forms of the species.

TABLE I

Part of the plant	Madras plant	Burmese plant, *C. nuda*	Bombay plant f. *kolhapurensis* Dixit.
Stem ..	434–476 μ broad	350 μ broad	325–350 μ broad
Branchlets ..	8–9	7–8	11
Bracteoles ..	½ as long as the oogonium	As long as the oogonium	Rudimentary
Antheridium ..	About 294 μ diam.	230 μ diam.	325–330 μ diam.
Oogonium ..	672–742 μ long × 504–518 μ broad	800 μ long × 525 μ broad	800–860 μ long × 425–450 μ broad
Coronula ..	126–140 μ height × 224–238 μ broad	70 μ in height × 190 μ in broad	130 μ in height × 250 μ in broad
Convolutions ..	10	14	10–12
Oospore ridges ..	10–12	12	Not given
Oospores ..	518–560 μ long × 364–392 μ broad	450 μ long × 330 μ broad	500 μ long × 280 μ broad

It may be seen from Table I that most measurements are overlapping each other among the different forms. The Madras plant differs from the type in the broader stems, larger number of branchlets, in the length of the bracteoles, the diameter of the antheridium, in the shorter oogonia and the larger coronula, in the number of convolutions being less, and also in the larger oospore. It also differs from the Bombay form in the thicker stems, shorter oogonia, smaller antheridia, in the smaller number of convolutions and in the larger oospores. The Madras plant occupies a middle position between the type and the form in the number of branchlets, in the length of the bracteoles, in the size of the gametangia and in the dimension of the coronula. It may also be seen that Pal (1932) does not give any variation in the different characteristics and measurements. It may after all prove that *Chara nuda* Pal may have a greater range of dimensions than has been mentioned by Pal and may lead to a conclusion that all the above plants are the same. This is an aspect which needs careful study. Until then we wish to keep the Madras plant as a separate form from the other two known.



TEXT-FIGS. 71–82. *Chara erythrogyna* & *C. flaccida*.

Figs. 71–75, 77, 78, 81. *C. erythrogyna*. Fig. 71. Habit. Fig. 72. A whole branchlet. Fig. 73. Branchlet node having oogonium only in a form from Malleswaram. Fig. 74. Stem node showing stipulodes and branchlets. Fig. 75. Oogonium. Fig. 77. Stem cortex and spine cells. Fig. 78. Branchlet node having the antheridium only. Fig. 81. Oospore. (Fig. 71, × ¾. Figs. 72, 74, × 10. Figs. 73, 78, × 22. Figs. 75, 77, 81, × 54.)

Figs. 76, 79, 80, 82. *C. flaccida*. Fig. 76. A whole branchlet. Fig. 79. Stem cortex. Fig. 80. Stem node showing stipulodes and branchlets. Fig. 82. Branchlet node with antheridium and oogonium. (Fig. 76, × 10. Fig. 79, × 86. Figs. 80, 82, × 17.)

Chara erythrogyna Griffith

(Text-Figs. 71–75, 77, 78, 81)

Griffith, 1847/9, p. 278; Groves, J., 1924, p. 372; Zaneveld, 1940, p. 166.

= *Chara griffithii* A.Br. in Braun and Nordstedt, 1882, p. 130.

Monœcious. Stem 490–574 μ thick; internodes 2–3 times the length of the branchlets; stipulodes in single row upto double the number of branchlets, elongated, with acute or acuminate apices; cortex diplostichous, cells of the primary series more prominent than the secondary. Spine cells short. Whorls of usually 11–13 branchlets. Branchlets very slender, entirely ecorticate, of 4–5 segments, lowest segment is the longest. Bracts-cells 4–6, long, with acute or slightly acuminate apices. Bracteoles 2–3 times as long as the fruit or longer. Gametangia usually situated at different nodes, the oogonia being commonly found in the upper nodes. Oogonia 490–742 μ long, 308–434 μ broad, spiral cells showing 9–10 convolutions, coronula usually converging towards the top or occasionally spreading, 84–112 μ high, 126–168 μ broad at the base. Oospore black, 462 μ long, 336 μ broad with about 8 ridges. Antheridium 280–308 μ in diameter.

Hab.: In a swamp at Malleswaram, Bangalore, Mysore State, April 1923; in a tank, at Talguppa, in Mysore State, 24–10–26 (All leg. M. O. P. I.)

Association: *N. oligospira* (Talguppa).

Allen (1928, p. 62) made an interesting observation on the distribution of the gametangia on the branchlets. He found an entire branchlet to be either male or female. The writer found similar instances. The Malleswaram form showed the normal arrangement of the gametangia, while the Talguppa material showed a feature similar to that recorded by Allen.

Bulbils were observed at the stem nodes in the Talguppa form.

Groves and Allen (1927, p. 339), Allen (1928, p. 62) and Zaneveld (1940, p. 167) favour the inclusion of *Chara thwaitesii* A.Br. under *C. erythrogyna* Griffith. *C. thwaitesii* was described by Braun (Braun and Nordstedt, 1882, p. 132) on a specimen from Cyelon collected by Thwaites. This resembles *C. erythrogyna* in its corticate stems and ecorticate branchlets and in the gametangia being solitary. But it differs in that the stipulodes are rudimentary, the number of branchlets vary from 10–12, and the branchlets have 4–5 segments. These characters are very variable characters and the species may be united with *C. erythrogyna*, this name having the priority.

The South Indian specimens of *C. erythrogyna* are interesting in that they show some points of difference with the type. The number of branchlets are 11–13 and the number of segments is 4–5, while in the type it is 13–16 branchlets and 6–8 segments. These characters are like those of *C. thwaitesii*. But unlike *C. thwaitesii* the stipulodes are not rudimentary. Thus it would appear that *C. erythrogyna* is a very variable species and the variation may be such that forms such as *C. thwaitesii* may easily be included in it.

The Malleswaram form differs from the Talguppa form in having larger fruits and also in having the coronula spreading. In the Talguppa form the coronula was tapering towards the top.

Chara flaccida A.Br.

(Text-Figs. 76, 79, 80, 82)

Braun, A., 1849, p. 296; Braun and Nordstedt, 1882, p. 128; Groves, J., 1924, p. 372.

= *Chara fibrosa* Ag. subsp. *flaccida* (A.Br.) Zanev.; Zaneveld, 1940, p. 162, Figs. 17 *a–c*.

Monœcious. Stem slender, 364–392 μ thick, internodes 1–2 times the length of the branchlets. Stem corticated, diplostichous, primary series more prominent than the secondary. Spine cells short. Whorls of 8–9 thick branchlets, with usually 4–5 segments. Stipulodes in single row and well developed, elongated. Bract-cells 5–7 (including bracteoles), thick. Gametangia produced at the lower three nodes and both oogonia and antheridia at the same node. Oogonia 574–630 μ long, 392–420 μ broad, spiral cells showing about 10 convolutions; coronula 112–126 μ high, 154–168 μ broad at the base, erect. Oospore golden brown, 462–490 μ long, 308–336 μ broad, with 8–9 inconspicuous ridges. Antheridia 392–448 μ in diameter.

Hab.: In a pool, near the edge of a tank, Tinnanur, near Madras, 5-6-23; in a pool (bog), Malleswaram, 24-12-23; in a tank at Guduvancheri, near Madras, 17-3-23; in Red Hills tank, near Madras, 21-2-25; in a tank, Singaperumal Koil, near Madras, 9-1-29; in a marsh at Ennore, near Madras, 8-2-40, 8-3-40; Vandalur, near Madras, 27-3-42 (All leg. M. O. P. I.).

Association: *C. zeylanica* and *N. hyalina* (Ennore) and *N. wattii* and *N. hyalina* (Guduvancheri).

The plants were very robust and presented a bristly appearance. In some of the specimens in the material, however, the colour of the oospore was dark brown.

The antheridia are larger than in the type and in this the present plants resemble the Bengal plant (*cf.* Agharkar and Kundu, 1937, p. 16).

The Singaperumal Koil form was incrusted with lime.

The South Indian plants agree generally with var. *wightii* A.Br. described by Braun from Madras.

Chara gymnopitys A.Br.

(Text-Figs. 85, 89–91)

Braun and Nordstedt, 1882, pl. 124; Groves, J., 1924, p. 373.

= *Chara fibrosa* Ag., subsp. *gymnopitys* (A.Br.) Zaneveld, 1940, p. 158.

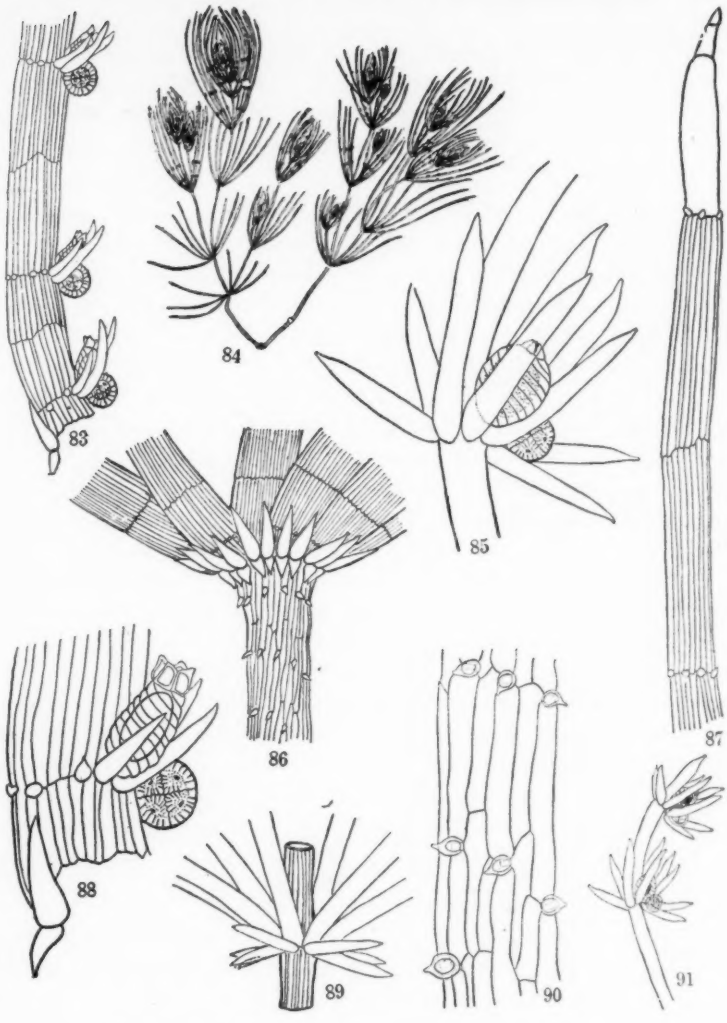
Monœcious. Stem slender, 238–266 μ thick; internodes 1–4 times the length of the branchlets; stem corticate, diplostichous, primary series more prominent than the secondary series. Spine cells solitary, short, 140 μ long. Stipulodes well developed, in a single row equal to the number of branchlets. Whorls of 7–10 branchlets. Branchlets of 3–4 segments, entirely ecorticate. Bract-cells 7–9 including bracteoles, long, exceeding the fruit in length, acuminate. Gametangia usually at the lowest two nodes, occasionally at the third node. Oogonium 420–434 μ long, 266–280 μ broad; spiral cells showing 9–10 convolutions; coronula short converging, 84 μ high, 140 μ broad at the base, Oospore black, 392–406 μ long, 280–294 μ broad with about 8 ridges. Antheridium 196–210 μ in diameter.

Hab.: In a tank, Vandalur, near Madras, Feb. 1923 and 1924; in a tank, Tirukalikundram, near Madras; in a tank, at Nagalapuram; in Alwar tank, at Tirupati Hills; January 1943 (All leg. M. O. P. I.).

Association: *C. zeylanica* (Tirupati Hills), *C. hydropitys* (Tirukalikundram).

The plants were slender and somewhat short and stunted.

Chara gymnopitys shows a very close resemblance to a few other species of *Chara*, *C. benthamii*, *C. flaccida* and *C. curtisii*. These species are distinguished by the number of stipulodes and the colour of the oospores. These characteristics are very variable and some of them can be noticed only in mature specimens. On similar grounds *C. benthamii* has often been recorded as a variety of *C. gymnopitys* (Groves, 1924, p. 373, also *cf.* Groves, 1927,



TEXT-FIGS. 83–91. *Chara brachypus & C. gymnopitys.*

Figs. 83, 84, 86–88. *C. brachypus.* Fig. 83. Lower portion of the branchlet. Fig. 84. Habit. Fig. 86. Stem node showing stipulodes and branchlets. Fig. 87. Upper portion of the branchlet. Fig. 88. The short lowest corticate segment. (Figs. 83, 86, 87, × 17. Fig. 84, × ⅔. Fig. 88, × 30.)

Figs. 85, 89–91. *C. gymnopitys.* Fig. 85. Branchlet node with antheridium and oogonium. Fig. 89. Stem node with stipulodes. Fig. 90. Stem cortex. Fig. 91. A whole branchlet. (Fig. 85, × 30. Fig. 89, × 17. Fig. 90, × 86. Fig. 91, × 10.)

p. 134 and Agharkar and Kundu, 1937, p. 18), though some others would like to keep this species distinct. Zaneveld (1940), on the same grounds of variability and also of the difficulty of observing the ripe oospore in immature plants, puts all these related species under one species and adopts the older name, *C. fibrosa* Ag.

Chara benthamii, according to Braun, differs from *C. gymnopitys* in the number of stipulodes and branchlets, the former having the same number of each, the latter double as many stipulodes as branchlets. Groves (1924, p. 373) is of the opinion that this character is a very variable feature and that many plants having an intermediate position have been recorded. Hence he treats *C. benthamii* as a variety of *C. gymnopitys*. Later the same author (1927, p. 134), again, reverts back to keep it distinct. He distinguishes *C. benthamii*, by the number of branchlets and stipulodes being generally the same, and by the constant number of bract-cells which are usually stout and short. He puts under *C. gymnopitys* all other forms which show variation to this definition. But still later (Groves and Allen, 1935, p. 57) a form which had only as many stipulodes as the branchlets is included. Zaneveld (1940, p. 154) differentiates *C. gymnopitys* from *C. benthamii* by the stipulodes, which are equal to the number of branchlets (8–14) and are longer and wider, and by the smaller oospores and larger oogonia. Yet in spite of all these distinctions Zaneveld would like to put it as a subspecies under *C. fibrosa*. The writer wishes to state that the differences are far too large and that such an amalgation of these species may lead large numbers of plants being jumbled together in one species without any specificity in the differentiation of the species. Hence he prefers to keep the species, *C. gymnopitys*, quite distinct from *C. benthamii* in consonance with the observations of Groves.

Zaneveld (1940, p. 160) distinguishes a number of varieties under *C. gymnopitys*.

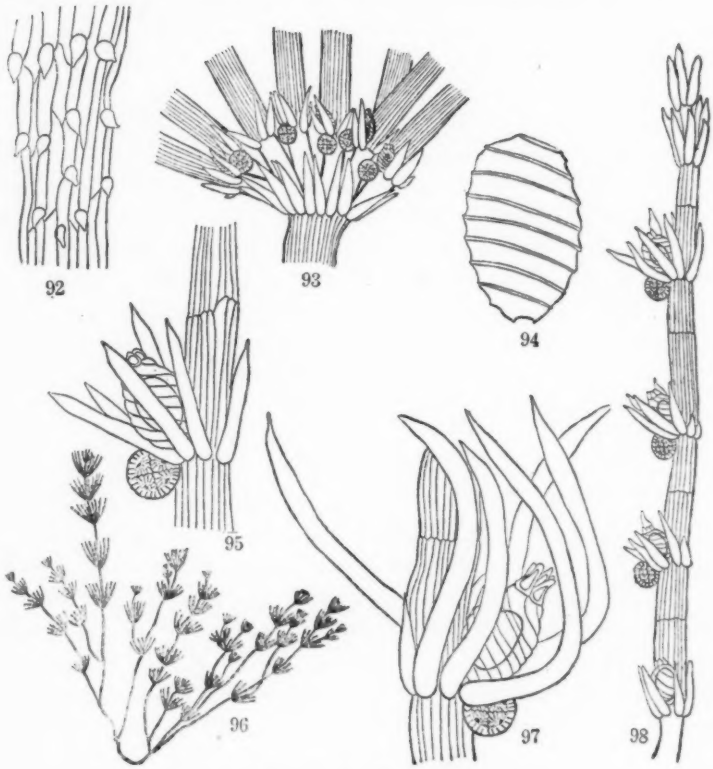
The present plant is clearly a *C. gymnopitys* and in the dimensions of the oospores and the bract-cells and also in the nature of the cortication, the present plants come under the var. *kurzii* nom. nov. [= var. *typica*, (A.Br.) Zanev.]. But it differs from the var. *kurzii* [= var. *typica* (A.Br.) Zanev.] in the smaller oogonia and in the smaller number of ridges, and convolutions.

Chara hydropitys Reichenbach ex Moessler

(Text-Figs. 92–98)

Braun, A., 1849, p. 297; Braun and Nordstedt, 1882, p. 133; Zaneveld, 1940, p. 168.

Monœcious. Stem rather slender, 336–434 μ thick; internodes as long as the branchlets or longer. Stipulodes in a single series, well developed, long, acute, double the number of branchlets. Stem cortex, diplostichous, primary cortical series more prominent than the secondary. Spine cells solitary, very small. Whorls of 8–11 spreading branchlets. Branchlets of 5–7 segments, the lowermost and the upper 2–3 segments ecorticate, the



TEXT-FIGS. 92–98. *Chara hydropitys.*

Fig. 92. Stem cortex. Fig. 93. Stem node showing stipulodes and branchlets. Fig. 94. Oospore. Fig. 95. Branchlet node with antheridium and oogonium. Fig. 96. Habit. Fig. 97. Branchlet node with antheridium and oogonium of *C. hydropitys* f. *plumosa*, f. nov. Fig. 98. A whole branchlet. (Fig. 92, × 54. Fig. 93, × 10. Fig. 94, × 180. Figs. 95, 97, × 36. Fig. 96, × ¾. Fig. 98, × 17.)

intervening segments corticate. Bract-cells 5–7, well developed, equal or slightly exceeding the fruit in length, bracteoles 2. Gametangia produced in the lowest 4–5 nodes. Oogonium 350–476 μ long, 224–308 μ broad;

spiral cells showing 9–11 convolutions; coronula 56–98 μ high, 112–140 μ broad at the base. Oospore black, 296–364 μ long, 196–266 μ broad, with 8–10 low ridges terminating in short basal claws. Antheridium 182–224 μ in diameter.

Hab.: On moist soil at the edge of a tank, at Vandalur, near Madras, February 1923 and 27–3–42; in a paddy-field at Adyar in Madras; in a paddy-field drain, at Singaperumal Koil, near Madras, 9–1–29; in a tank, at Tirukalikundram, Madras State (All leg. M. O. P. I.).

Association: *C. nuda* (Singaperumal Koil). *C. gymnopitys* (Tirukalikundram).

These plants were heavily incrusted with lime and were 2–10 cm. in height. Plants of this species, collected from Adyar, were 1–2·5 cm. in height and were growing in small clumps.

The different specimens collected from South India vary from each other very much. A number of varieties are known under this species. Braun and Nordstedt (1882, p. 133) distinguish them according to the number of branchlets, the number of corticated segments and the length of the oospore. The different varieties are *indica*, A.Br., *hydropitys* nom. nov. (= var. *genuina*, A.Br.), *perfecta*, A.Br., *majuscula*, Nordst., *africana*, A.Br. and *brachypitys*, A.Br. So far only var. *indica* has been recorded from India.

Specimens collected from three localities, Seven Pagodas, Tirukalikundram and Singaperumal Koil resembled very closely var. *indica*. There is a general agreement in several aspects. The oospore measurements showed a large variation. The Seven Pagodas form showed a few more interesting features. It had very long and curved bracts and bracteoles and the coronula is very much elongated. It is described as a distinct form under var. *indica*.

(*a*) Var. *indica*. A. Braun, 1849, p. 296; Braun and Nordstedt, 1882, p. 135; Allen, T. F., 1888, p. 56; Zaneveld, 1940, p. 171.

forma *minor* A. Braun, 1849, p. 297.

Plants upto 5 cm. high. Stem 280–322 μ thick, internodes as long as or little shorter than the branchlets. Cortex, diplostichous, primary row of cortical cells more prominent than the secondary row. Branchlets 9, straight, with 6 segments, the lowest and the upper 2 segments ecorticate, the rest corticate. Stipulodes single row, well developed. Spine cells, solitary, rudimentary. Bracts and bracteoles 8. Gametangia produced in the 2nd to the 5th nodes. Antheridia, 196–210 μ in diam. Oogonia

336–350 μ long and 210–224 μ broad with the spiral cells showing 10 convolutions; coronula 56–70 μ high and 112–126 μ broad at the base. Oospores black, 308–350 μ long and 224–238 μ broad.

Hab.: In a tank at Tirukalikundram, Madras State.

This form is characterized by the extremely small size. The plant was recorded by Braun from a collection of Belanger from the Coromandel Coast, made in 1826–28.

forma *major* A.Br., 1849, p. 297; Zaneveld, Charophyta of Malaysia, 1940, p. 173, figs. 18 *a–c*.

Plants 7·5–12·5 cm. high. Stem 336–392 μ thick, internodes as long as the branchlets, or slightly longer. Cortex diplostichous, primary rows more prominent. Branchlets 10–11, straight, with 5–6 segments, the lowest and the upper 2–3 segments ecorticate. Stipulodes single row, well developed. Spine cells, solitary, rudimentary. Bracts and bracteoles 8–9. Gametangia produced from the 2nd to the 5th nodes. Antheridium 210–238 μ in diameter. Oogonium 406–420 μ long and 294–308 μ broad, ellipsoidal, spiral cells showing 9–10 convolutions; coronula 56–70 μ high and about 126 μ broad at the base. Oospore black, ellipsoid cylindrical, 350–364 μ long and 252–266 μ broad.

Hab.: In a paddy-field drain, at Singaperumal Koil, near Madras, 9-1-29.

This form differs from var. *indica* f. *minor* only in the larger habit. This plant from Singaperumal Koil agrees with the forma *major* in all respects, except in that the oospore is slightly broader and in the number of convolutions being smaller. This plant was heavily incrusted with lime. This form also resembles var. *hydropitys* in the slightly larger dimensions of the oospores.

One other specimen of this species collected from Seven Pagodas was very interesting, as has been stated earlier. It agrees with var. *indica* f. *major* in all respects, except in (1) the bracteoles are 2–3 times as long as the fruit so that some sort of a feathery appearance is given to the plant, (2) in the longer coronula cells (112–140 μ high and 128–140 μ broad as the base) and (3) in the presence of the gametangia on the 6th node. The oogonia are also longer (518–546 μ) than in the other plants and have 10–11 convolutions. This plant is placed in a new form under var. *indica* as f. *plumosa*, f. nov.

f. *plumosa*, f. nov.

Plants 7·5–10 cm. in height. Stem 378–406 μ thick; internodes as long as branchlets. Branchlets 9, straight, segments 7, the lowermost and upper 2–3 segments ecorticate, bracteoles 2–3 times as long as fruit. Gametangia on the 2nd to the 5th node, sometimes on the 6th also. Antheridia 182–196 μ in diameter. Oogonia 518–546 μ long and 280–294 μ broad, ellipsoid with 10–11 convolutions; coronula 112–140 μ high and 128–140 μ broad at the base. Oospore about 308 μ long and 196–210 μ broad with 9–10 low ridges terminating in basal claws. (Text-Fig. 97).

Hab.: In a pool at Seven Pagodas, Madras State (Leg. M. O. P. I.).

One collection of *C. hydropitys* which was made from Vandalur generally agreed with the var. *indica* A.Br. and also with var. *africana* A.Br., in the dimensions of the oospores and in the number of segments. But whorls of 8–9 and sometimes a whorl of 10 branchlets were also met with. Judging it from the number of branchlets it comes under var. *africana* and similarly if we take into account the presence of 10 branchlets it can also be put under var. *indica*. Thus it would seem as if that the method of division or demarcation based on the number of branchlets seems to be not so useful, and often presents difficulty in such instances as has been pointed out here. Similar difficulty was met with in a form of *C. hydropitys* collected from paddy-fields at Adyar, in Madras. This alga agreed very closely with var. *indica* but had 8–9 branchlets and the number of segments was 5. Based on these two factors it comes under the var. *africana*. But it differed from var. *africana* in the length of the oospore and in the dimensions of the oogonium which was smaller. It further resembled var. *indica* f. *gymnophylla* in the occasional presence of entirely ecorticate branchlets. A study of these two specimens generally tend to show that the demarcation of the var. *indica* and var. *africana* is not very sound and warrants a combination of these varieties putting those that have the forms like var. *africana* as a separate form under the same for purposes of convenience. A perusal of table of measurements (Table II) will show that the characteristics of these two varieties and those of the different South Indian specimens are coextensive and naturally merge into one another through intermediate forms. No doubt these forms show an affinity to the var. *brachypitys* but the very large oospores will help to clearly demarcate the variety.

The Adyar form was further interesting in that occasionally a whole branchlet was either bearing antheridia or oogonia, though they are found together very commonly.

TABLE II

	Var. *indica* A.Br.	S. P. Koil	Tirukali-kundrum	Seven Pagodas	Vandalur	Adyar	Var. *africana* A.Br.
Branchlets ..	9–13	10–11	9	9	8–9	8–9	8–9
Segments ..	6–7	5–6	6	7	6–7	5	5–6
No. corticate segments	3–4	2–3	3	3–4	3–4	0–2	1–2
Oospore	280–350 μ × 220–260 μ	350–360 μ × 252–266 μ	308–350 μ × 224–238 μ	308 μ × 196–210 μ	336–350 μ × 226–238 μ	294–336 μ × 190–238 μ	330–380 μ × 190–220 μ

Chara brachypus Braun

(Text-Figs. 83, 84, 86–88)

Braun, A., 1849, p. 298; Braun and Nordstedt, 1882, p. 185; Groves, J., 1924, p. 375; Zaneveld, 1940, p. 199, figs. 15 *a–d*.

Monœcious. Stem 574–742 μ thick; internodes as long as or shorter than the branchlets. Stipulodes in double rows, well developed, acute. Stem cortex triplostichous, cells of the primary and secondary series equally prominent. Spine cells solitary, small, acute. Branchlets 9–11 in a whorl, incurved, with 9–10 segments, the lowest segment is very short; the ultimate 2–3 segments ecorticate, the rest corticate, cortical cells of the branchlets about three times as many as the bract-cells. Bract-cells 6–8, unequally developed; bracteoles as long as or slightly longer than the oogonium. Gametangia produced at the three lowermost nodes. Oogonium 560–728 μ long, 350–392 μ broad; spiral cells showing about 12 convolutions; coronula 126–154 μ high, 168–204 μ broad at the base. No ripe oospores seen. Antheridium 308–350 μ in diameter.

Hab.: In a pool, Seven Pagodas; in a pool near the edge of a tank at Tinnanur, near Madras, 5–6–23 (All leg. M. O. P. I.)

Association: *C. braunii* and *Nitella oligospira* (Seven Pagodas).

The oogonia in the present forms are somewhat shorter than the type. There is a close agreement in the dimensions of the other parts. The South Indian plants differ from the rest in that some of the apical segments of the branchlets are often ecorticate (*see* Agharkar and Kundu, 1937, p. 18).

Both the collections had a heavy incrustation of lime.

Two varieties of *C. brachypus* are known, var. *gracilescens*, A.Br. and var. *ehrenbergiana*, A.Br. The former was recorded from Madras

(A. Braun, 1849, p. 298) and the latter from Egypt (A. Braun, 1868, p. 867). Zaneveld (1940, p. 202) differentiates the two varieties as follows: "Var. *gracilescens* is probably a slender form with less than 8–9 branchlets, inconspicuous spine cells, whereas the uncoloured lowest articulation is longer than the stipulodes. Var. *ehrenbergiana* is somewhat aberrant as the branchlets in the lower whorls and in some of the upper ones are entirely ecorticate. Most probably it is a monstrosity." The Madras forms have 9–11 branchlets and the stipulodes are well developed and are longer than the 1st segment of the branchlet. In these characters these specimens differ from var. *gracilescens*, the variety originally recorded from Madras. Also, the dimensions of the gametangia are smaller than those of the type. They do not agree with the var. *ehrenbergiana* in the absence of entirely ecorticate branchlets. The Madras plants agree very much with the Bengal plant, described by Agharkar and Kundu (1937, p. 17).

In the absence of cortication in the upper 2–3 segments, long, sharp stipulodes, and acute spine cells, this form comes very near *C. pseudobrachypus* Groves and Stephens (1926, p. 156), which differs from *C. brachypus* "in the diplostichous branchlet cortex and less abbreviated lowest branchlet segment". It would appear that *C. pseudobrachypus* is based on the length, or, in other words, the lack of shortening of the first segment of the branchlets, the larger oogonia, and the presence of ecorticate ultimate segments. The last of the characters is also shared by *C. inermis* Zaneveld, and by *C. zeylanica*.

These Madras forms come very closely to *Chara inermis* Zaneveld (1940, p. 198) described from Malaysia. This species of Zaneveld differs from *C. brachypus* essentially in the absence of the spine cells, and also in the smaller oospores. It is of interest that the Madras plants agree very well with this species, in all the dimensions, in the ecorticated nature of the terminal segments of the branchlets, and in the well-developed stipulodes. In this connection it may be pointed out that Zaneveld remarks (1940, p. 201) that *C. brachypus* can be "distinguished by the presence of spine cells, the *rudimentary stipulodes* and the larger gametangia" (italics author's). The stipulodes in *C. brachypus* are not rudimentary, only the upper row is better developed than the lower row. For that matter if one compares the measurements of the stipulodes given by the author himself for the two species, one is unable to perceive much difference in the length of the stipulodes (*C. brachypus*, upper row, $600 \times 60\,\mu$, lower row $225 \times 100\,\mu$; *C. inermis*, upper row, $525 \times 95\,\mu$ and the lower row $315 \times 60\,\mu$). Thus it settles down to this that Zaneveld's species differs from *C. brachypus* in the absence of spine cells (*see* Zaneveld, 1940, p. 121).

Chara zeylanica Willd.

Willdenow, 1896, p. 59, pl. 2, fig. 1, Groves, J., 1924, p. 375; Zaneveld, 1940, p. 203, figs. 21 *a–d*.

Monœcious. Stem stout, 700–850 μ thick; internodes 1–2 times the length of the branchlets. Stipulodes in 2 rows, twice as many as branchlets, well developed, elongated, acute; cortex triplostichous; cells of the primary and secondary series equally prominent; spine cells solitary, elongated acute, sometimes acuminate. Branchlets 9–12 in a whorl, spreading; segments 9–11, the lowermost segment very short and ecorticate, the ultimate 1–2 segments sometimes ecorticate, other segments corticate, triplostichous. Bract-cells well developed, about 8, exceeding the oogonium in length. Gametangia produced from the 2nd to the 4th nodes, sometimes also in the 5th node, occasionally on the first branchlet node also. Oogonium ovate, 800–896 μ long, 448–504 μ broad, spiral cells showing 10–12 convolutions; coronula slightly spreading, 126–140 μ high and 210–224 μ broad. Oospore brownish to black, 560–700 μ long and 350–392 μ broad, with fine granulations. Antheridium 420–448 μ in diameter.

Hab.: Common in many tanks, ponds, beach pools around Madras; in a tank at Guduvancheri, near Madras, 17–3–23; in a roadside ditch near the edge of a tank, Singaperumal Koil, near Madras, 9–1–33; in a tank at Tiruvellore, 21–1–33; in a lake at Nagalapuram, Chittoor District, January 1933; in a well, Krusadai Island, 5–4–37; in a tank at Pamban, 8–10–37; 8–10–40; in a pool at Tinnanur, near Madras; in a lake at Chingleput, near Madras, November 1937; in a running stream, Ralla Kalava, near Renigunta, Chittoor District, 11–1–41; in a pool and a tank at Vandalur, near Madras, February 1923, 17–3–23, 16–1–25; in a tank at the Tirumalai Hills at Tirupati, Chittoor District, January 1943; in a swamp at Ennur, 8–7–41 (All leg. M. O. P. I.).

Chara zeylanica has been recorded from a large number of places in South India. This species is an extremely variable one. A. Braun has created a large number of varieties under *Chara zeylanica*. Later Allen (T. F.) has described them variously as varieties and also as distinct species. Robinson (1906, p. 291) followed Allen's example and created a few more new species of his own. Groves, H. & J. (1912, p. 41) do not recognise them as separate species or even as varieties. But they consider them merely as forms. They further state, that all the West Indian specimens which they examined 'appear to be more or less intermediate, rarely agreeing exactly with any of the named forms'. Zaneveld (1940, p. 204) working on the Malaysian specimens of *Chara zeylanica* differentiates them into

two distinct categories of plants, according to whether the branchlets, spine cells and bract-cells are shorter or longer, *i.e.*, macroscopically visible or not. In the first he puts f. *typica* including in it the type of the species, and the var. *ceylonica* of A. Braun and in the latter he creates two forms, f. *armata* including part of the old var. *armata* A.Br. and f. *diaphana*. This last form is differentiated from the second by having more than four ecorticate branchlet-articulations or sometimes entirely ecorticate branchlets and by the fertility of the first branchlet node. Zaneveld, however, does not give any opinion regarding the other varieties of *zeylanica*.

Groves (1931; *see also* Zaneveld, 1940; Dixit, 1940) has shown that the antheridium of *C. zeylanica* is quadriscutate instead of being octoscutate, as is generally the case in the charophytes. Sundaralingam and Francis (1958) have confirmed the observation of Groves and corroborated it by studying the development of the antheridium in *C. zeylanica*, a quadriscutate species, and *C. corollina*, an octoscutate one. No other species of *Chara* has so far been reported to have a quadriscutate condition. In the light of these observations, inclusion of the different species of Allen and Robinson under *C. zeylanica* can only be tentative and any firm decision must await a study of the antheridia in the different species or varieties sought to be included in *C. zeylanica* either based on type collections or on topotype collections.

With this background the writer made an attempt to see if the different specimens can be segregated into the different varieties, or forms. In this he is in agreement with Groves, H. & J. in regarding them as different forms. It may be pointed out at the outset that very often it was difficult to put a particular form merely by the key given by either Braun or Allen. Still, these form the only valuable guides for the identification of the different forms. As many as five forms have been distinguished, f. *macilenta*, f. *inconstans*, f. *hildebrandtiana*, f. *humboldtii* and f. *curassavica*.

(*a*) f. *macilenta* (A.Br.) comb. nov.

= *Chara zeylanica* var. *macilenta* A.Br., Braun and Nordstedt, 1882, p. 192, pl. 7, fig. 271, Allen, T. F., 1888, p. 63.

Stem very variable in thickness, 350–700 μ. Spine cells, short and 70–260 μ in length, tapering. Stipulodes of the upper row well developed and hide almost or completely the lower segment; stipulodes of the lower row up to or as long as those of the upper. Branchlets 10–11; segments 8–11, the lowermost segment a little longer than the diameter of the branchlet and very rarely 1–2 times as long as the diameter, ending in a mucro; in some the last segment

ecorticate. Bracts, anterior ones slightly longer than the oogonium, mostly or rarely 1½ times as long as the oogonium. Gametangia on all the nodes of the branchlets including the 1st node. Antheridium very variable in diameter 210–336 μ. Oogonium 434–602 μ long, 154–280 μ broad, coronula generally slightly spreading, very rarely erect, 84–126 μ high and 112–168 μ broad at the base. Oospore 420–588 μ long and 266 μ broad.

Hab.: In a tank at Vandalur, near Madras, 17–3–23; in a hill pool in the same locality, Feb. 1923; in a roadside ditch at the edge of a tank at Singaperumal Koil, near Madras, 9–1–33; in a tank at Guduvancheri near Madras, 17–3–23.

The South Indian specimens agree with Braun's description of the var. *macilenta*, in the nature of the stipulodes, the length of the anterior bracts, the dimensions of the antheridium, oogonium and the oospore, in the spine cell being short and tapering and in all the branchlet nodes being fertile. But these differ from the description of the variety in that none of the apical segments except the end mucro is ecorticate. In this aspect these resemble the var. *ceylonica* of A. Braun but differ from it in the dimensions of the oogonia. These also differ in the number of branchlets in a whorl being 10–11 as against 8–9 in the type.

(*b*) *f. inconstans* (Kütz.) Groves, H. & J., 1912, p. 41.

= *C. inconstans* Kützing, *Tab. phycologicæ*, 1857, 28, pl. 70, fig. 2.

= *C. ærstediana* Braun, 1857/1858, p. 367.

= *C. gymnopus* Braun in Braun and Nordstedt, 1882, p. 193.

Stem very variable, 280–588 μ in thickness. Stipulodes of the upper row more strongly developed, concealing the lowest node or a little shorter than the lowest branchlet node, of the lower row ½ as long as those of the upper row. Branchlets 8–10 with 7–8 segments, the lowest segment equal to or a little longer (1–2 times longer) than the diameter of the branchlet; at the apex besides the tip, which is a mucro, nothing ecorticate. Spine cells short, acute, 56–168 μ long. Bract cells, anterior ones slightly longer or 1½–2 times as long as the oogonium. Gametangia at all nodes including the first. Oogonium 420–560 μ long, 154–294 μ broad; coronula short, erect or slightly spreading, 70–126 μ high and 126–154 μ broad at the base. Oospore black, 420–560 μ long and 252–378 μ broad. Antheridium 196–336 μ in diameter.

Hab.: In a tank at Vandalur, near Madras, Feb. 1923; 16–1–25; in a tank at Tiruvellore, 21–1–23.

These specimens agree very closely with Braun's description of var. *inconstans*, f. *crucigeriana* in the thickness of the stem, in the stipulodes being shorter than the first leaf node, in having 6–8 nodes, in the apex of the branchlet having a naked mucro, in the anterior bracts double as long as or equal to the oogonium, in the diameter of the antheridium, in the dimensions of the oospore and the fertility of all the branchlet nodes. They differ in having shorter spine cells, in which aspect they resemble f. *ærstediana*. These specimens further differ from the type description of var. *inconstans* in the absence of ecorticate segments in the upper portion of the branchlets.

(*c*) f. *hildebrandtiana* (A.Br.) comb. nov.

= *C. gymnopus* var. *hildebrandtiana* A.Br., Braun and Nordstedt, 1882, p 196.

Stem 400–672 μ thick. Stipulodes of the upper row more strongly developed than the lower row and concealing or a little shorter than the lowermost branchlet node. Branchlets 10–12 with 8–12 segments, the tip a naked mucro; lowest branchlet segment twice as long as the diameter of the branchlet, though in one specimen some were about 1½ times as long as the diameter of the branchlet. Anterior bracts equal to or a little longer or 1½–2 times as long as the oogonium, posterior bracts elongate, stiff and tapering. Spine cells numerous elongated and sharply pointed, 434–616 μ long. Gametangia on the second and third nodes. Oogonium 490–672 μ long and 210–280 μ broad (though in one specimen it was 616–672 μ long and 126–182 μ broad); coronula short, 70–98 μ high (rarely 126–182 μ high), erect and slightly spreading with conically elongated tips. Oospore 602–812 μ long and 336–462 μ broad. Antheridium 336–434 μ in diameter.

Hab.: In a pond at Chepauk, in Madras, 11–1–21; in a pond at Pamban, 8–10–37, 8–10–40.

These plants agree very closely with Braun's description of var. *hildebrandtiana*.

One form collected from a pond at Chepauk in Madras (11–1–21) was very interesting. In the sterile lower node, the dimensions of the oospore and in the length of lowest segment of the branchlets it agrees with the form. But differs from it in that 1–2 apical branchlet segments are ecorticate.

(*d*) *f. humboldtii* (Kütz.) Groves, H. & J., 1912, p. 42.

= *Chara ploiphylla* var. *humboldtii* Kützing, *Species Algarum*, 522, 1849.

= *Chara gymnopus* var. *humboldtii* Braun, in Braun and Nordstedt, 1882, p. 196.

Stem 378–602 μ thick. Spine cells shorter than the diameter of the stem, sometimes longer. Stipulodes, upper row more strongly developed than the lower row concealing the lowermost segment. Branchlets (8–) 9–11 (–12), with (7–) 9–11 (–12) segments, lowermost segment equal to or a little shorter or a little longer than the diameter of the branchlet, each branchlet ending in a mucro, the upper 1–2 segments usually ecorticate. Bracts, anterior ones 1½–2 times as long as the oogonium or rarely a little longer. Gametangia at the 2nd to the 4th nodes, sometimes at the 5th node. Oogonium 462–686 μ long and 140–294 μ broad, coronula slightly spreading or well spread, rarely erect, 84–168 μ high, (98–) 126–210 μ broad at the base. Oospore (532–) 602–756 μ long and 280–490 μ broad. Antheridium 252–462 μ in diameter.

Hab.: In a tank, at Red Hills, Madras, 21–2–25; in a tank at Chepauk, Madras, 17–3–23, 19–3–23, Sept. 1928; in a brackish water well, Krusadai Island, Madras State, 5–4–37; a lake at Chingleput, Nov. 1937; in a lake at Nagalapuram, Jan. 1933; in a running stream, Ralla Kalava, near Renigunta, 11–1–41.

Var. *humboldtii* of A. Braun is generally characterised by the lowest segment of the branchlets being equal to or a little longer than broad. But in two collections, *viz.*, from Renigunta and from Nagalapuram, the lowermost segment is shorter than the diameter of the branchlet. The number of apical segments which are ecorticate is very varying. While generally 1–2 segments are ecorticate in some even three segments are ecorticate (Krusadai Island, April 1937 and Madras specimens, Sept. 1928).

(*e*) f. *curassavica* (A. Br.) Groves, H. & J., 1912, p. 42.

= *Chara gymnopus* var. *curassavica* Braun ex Braun and Nordstedt, 1882, p. 198, fig. 105.

Stem 350–518 μ thick. Spine cells short, acute, upto ½ as long as the diameter of the stem. Stipulodes of the upper row more strongly developed than the lower concealing the lowest segment. Branchlets 8–12 with 8–9 segments in each, lowest segment 1½–2 times as long as the diameter, the tip a naked mucro, the last 1–2 segments, rarely 3, ecorticate. Anterior bracts a little longer or twice as long as the oogonium. Gametangia on the second to the fourth branchlet nodes, occasionally on the first. Oogonium 448–616 μ long and 154–252 μ broad, coronula slightly spreading. Oospore 560–644 μ long and 322–406 μ broad. Antheridium 322–392 μ in diameter.

Hab.: In standing water in the upper reaches of Adyar River, Madras; in a tank at Red Hills, Madras, 31–8–29.

Two collections have been made from South India which resembled f. *curassavica* (A.Br.) Groves, H. & J. In the number of branchlets and in the number of segments, the length of the basal segment, the elongated anterior bracts and in the shorter spine cells this form resembles very closely var. *curassavica*. Groves, H. & J. state that the length of the first branchlet segment is 2–6 times the diameter, whereas in the limited material that I examined I found it only upto 2 times the diameter of the branchlet. In many of these features, these specimens also resemble the var. *ceylonica* A. Braun, but it is very difficult to identify these forms very definitely on the basis of colour, especially from the formalin material. With var. *ceylonica* these similarly resemble in general but differ in the number of branchlets being less, in the dimensions of the oospore, and in the smaller number of ecorticate segments. Among these especially the material from Red Hills resembles the var. *ceylonica* in the very short spine cells (56–112 μ long).

Chara vandalurensis sp. nov.

(Text-Figs. 99–103)

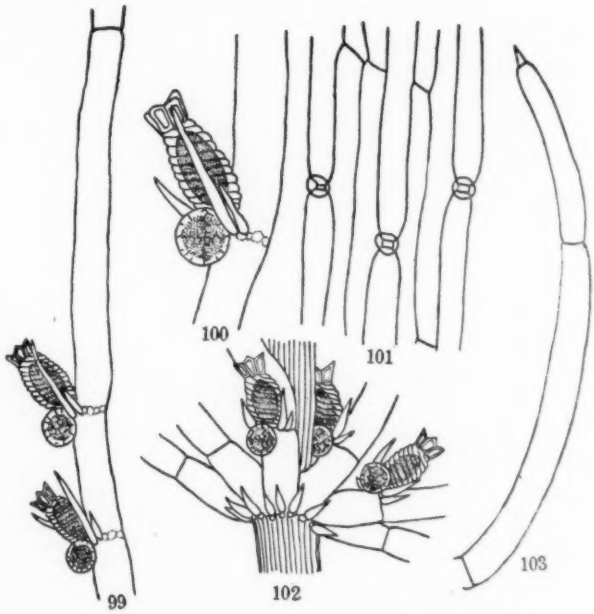
Plant monœcious, incrusted with lime. Stem moderately thick, about 400 μ thick, triplostichous, primary rows slightly broader than the secondary ones. Branchlets ecorticate, 10–11 with 5 segments, the first two segments very short and the 3rd and 4th segments longest, ending in a mucro. stipulodes 2 rows, upper row acute and well developed, the lower row very rudimentary. Spine cells, rudimentary, oblong in surface view with rounded edges. Bracteoles and bracts, 4, seen in the lower 2 nodes, all curved inside, bracteoles less than half as long as the oogonium, occasionally longer. Gametangia together, at the lowest 2 branchlet nodes, occasionally also at the third node. Oogonia 560–644 μ long and 266–280 μ broad, spiral cells showing about 12 convolutions, coronula 126–154 μ high and 168–182 μ broad at base. Oospore brown, 420–462 μ long and 308–322 μ broad. Antheridia 308–322 μ in diameter.

Hab.: In a pool at Vandalur, near Madras, Feb. 1923 (Leg. M. O. P. I.).

This species was collected only once and has since not been collected from either the same or any other place.

There are at the present moment two species among the triplostichous, diplostephanous *Charas* with ecorticate branchlets, *C. handœ* Pal and *C. guatemalensis* (Nordst.) Robins. The latter species was created by Robinson (1906, p. 286) from *C. gymnopus* var. *guatemalensis* Nordst. The former was created by Pal (1932, p. 86) from a plant collected from Burma. The

present form differs from *C. handæ* in the lower row of stipulodes being rudimentary, in the first two segments of the branchlets being very short, in the bracteoles being shorter than the oogonium, and in the spine cells being rudimentary. Further, oospores are smaller in the present form and the number of convolutions of the oogonium less. The branchlet ends in a mucro. Hence this plant is kept as a separate species. This agrees with *C. guatemalensis* in the second row of stipulodes being rudimentary, but differs from it in the larger number of branchlet segments, in the lowermost nodes being fertile, in the lowermost two segments being very short and also in the smaller gametangia.



TEXT-FIGS. 99–103. *Chara vandalurensis* sp. nov.

Fig. 99. First and second branchlet nodes with gametangia. Fig. 100. Branchlet node showing antheridium and oogonium. Fig. 101. Stem cortex. Fig. 102. Stem node showing stipulodes and branchlets (only 6 branchlets shown). Fig. 103. Upper portion of branchlet. (Figs. 99, 102, 103, × 22. Fig. 100, × 30. Fig. 101, × 86.)

LATIN DIAGNOSES

Chara vandalurensis sp. nov.

Planta monoica, incrustrata calce. Culmis sat crassus, ca. 400 μ crassus, triplostichus, lineis primariis paulo latioribus quam secundariis. Ramuli

10–11, segmentis 5; segmenta duo prima brevissima, tertium et quartum longissima, desinentia in mucronem. Stipulodiorum series duplex, quarum superior acuta et bene evoluta, inferior vero inchoata, aspectu superficiali oblonga, marginibus rotundatis. Bracteæ et bracteolæ 4, clareæ ad nodos 2 inferiores, omnes intus curvatæ, bracteolæ plus dimidio breviores oogonio, raro longiores. Gametangia simul ad 2 infimos ramulorum nodos, non-numquam etiam ad tertium nodum. Oogonia 560–644 μ longa, 266–280 μ lata, cellulis spiralibus duodecies convolutis, coronula 126–154 μ alta, 168–182 μ lata ad basim. Oosporæ brunneæ, 420–462 μ longæ, 308–322 μ latæ. Antheridia diametientia 308–322 μ.

Typus lectus in palude ad Vandalur proper urbem Madras, mense februario anni 1923.

Chara hydropitys var. *indica* forma *plumosa* forma nova.

Plantæ 7·5–10 cm. altæ. Culmis 378–406 μ crassus; spatia internodalia æque longa ac ramuli. Ramuli 9, recti, segmentis 7, quorum infimum et superiora 2–3 ecorticata, bracteolis bis terve longiores fructu. Gametangia ad nodos secundum ad quintum, non-numquam etiam ad sextum. Antheridia 182–196 μ diam. Oogonia 510–546 μ longa, 280–294 μ lata, ellipsoidea, convolutionibus 10–11; coronula 112–140 μ alta, 128–140 μ lata ad basim. Oospora ca. 308 μ longa, 196–210 μ lata, ornata 9–10 jugis parvis, desinentibus in ungues basales.

Typus lectus in palude ad Seven Pagodas, in Statu Madras.

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STUDIES IN THE ORDER PIPERALES

V. A Contribution to the Study of Floral Morphology of Some Species of *Piper*[1,2]

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(Communicated by Prof. V. Puri, F.A.SC.)

Introduction

Johnson (1902) was perhaps the first to investigate *Piper* (*P. medium*) and report an 8-nucleate, *Adoxa*-type of embryo-sac developing directly from an archesporial cell. This was confirmed by him in 1910 from his study of *P. betel* var. *monoicum*, and by Fisher (1914) from his study of *P. tuberculatum*. However, Schnarf (1931) from a close scrutiny of the diagrams suspected that the embryo-sac should be other than the *Adoxa*-type. That this suspicion was well founded, was shown by Swamy (1944) who, on a reinvestigation of *P. betel*, found the development to follow *Fritillaria*-type. In the same year Joshi reported the same type of embryo-sac development in *P. longum*.

Some difference of opinion also exists regarding the number of carpels in *Piper* gynæceum. Whereas Baillon (1874) considered it as monocarpellary, Eckardt (1937) regarded the same as psudomonomerous and tricarpellary.

The present of the series of papers deals with the floral anatomy of 12 species and embryology of one species of *Piper*.

Material and Methods

Preserved material of *P. Gaudichaudianum* and *P. Tucumanum* was obtained from Dr. T. Meyer of Argentina; that of *P. betle* from Mr. Y. K. Murty; *P. Schmidtii* from Mr. A. N. Rao; *P. nigrum* and *P. longum* from Prof. P. Maheshwari; *P.* spp. from Dr. K. M. Silberschmidt of Brazil and *P. subrubrispicum* from Prof. V. Puri and paraffin embedded material of *P. obtusilimbum*, *P. unguiculatum* and *P. nigrescens* from Dr. O. Hagerup.

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[1] Based on a portion of a thesis accepted for a Ph.D. Degree of the Agra University.

[2] Research contribution No. 18 from the School of Plant Morphology, Meerut College, Meerut.

Material of *P. longum* and *P. subrubrispicum* was also collected by the author from Saharanpur and Dehra Dun respectively. Serial transverse and longitudinal sections, 10–12 microns thick, were cut and stained with crystal violet and erythrosin.

Observations

The flowers may be unisexual (*P. Schmidtii*, *P. Wightii*, *P. nigrum*, *P. longum*) or bisexual (*P. Gaudichaudianum*, *P. Tucumanum*, etc.) and occur in leaf opposed cylindrical spikes. With unisexual flowers the plants are diœcious. Occasionally the female spikes of *P. nigrum* and the male spikes of *P. longum* were observed with some bisexual flowers. The male spikes in general are narrower, longer and more compact than the female ones except in *P. Schmidtii* where they are shorter.

Each flower is subtended by a peltate bract, which has a prominent stalk (*P. Gaudichaudianum* and *P. obtusilimbum*). The bract of *P. nigrum* is adnate to the inflorescence axis with which it forms a minute cup round the ovary. Hooker (1885) probably mistook this cup to be bracteoles. Often in *P. Gaudichaudianum* the stamens are adnate to the ovary wall for about half way up. In *P. Tucumanum*, however, they separate only just below the stigma, while in *P. longum* the fusion of the flowers is so complete that it involves all parts except the stigmas and apices of bracts which are free and which project out from the surface of the spike (Fig. 1).

The flowers are many, sessile, naked and arranged spirally on the axis. Male, female and bisexual flowers are sometimes found in one and the same species as *P. longum*. The male flower has two (*P. Schmidtii*) or three (*P. longum*, *P. unguiculatum*, etc.) stamens and a pistillode, while the female flower has a tricarpellary gynæceum with three sessile stigmas and two (*P. Wightii*, Fig. 2) or three (*P. nigrescens*) staminodes. In the hermaphrodite flowers there are two (*P. subrubrispicum*) or three (*P. Gaudichaudianum* and *P. Tucumanum*) stamens. The filaments are long and jointed in *P. Gaudichaudianum* and *P. Tucumanum*. In the latter species the constriction is just above the point of its separation from the ovary wall.

Ordinarily a single ovule occupies the ovarian cavity, but in one instance, second rudimentary ovule was observed in *P. Wightii* (Fig. 3). The fruit is a one-seeded drupe with a hard endocarp.

Development of the Bract.—The development of the bract in general is similar to that in *Peperomia* (Murty, 1958). The bract arises as a small protuberance caused by the activity of the sub-epidermal cells of the apex of the inflorescence axis. This protuberance elongates towards the apex

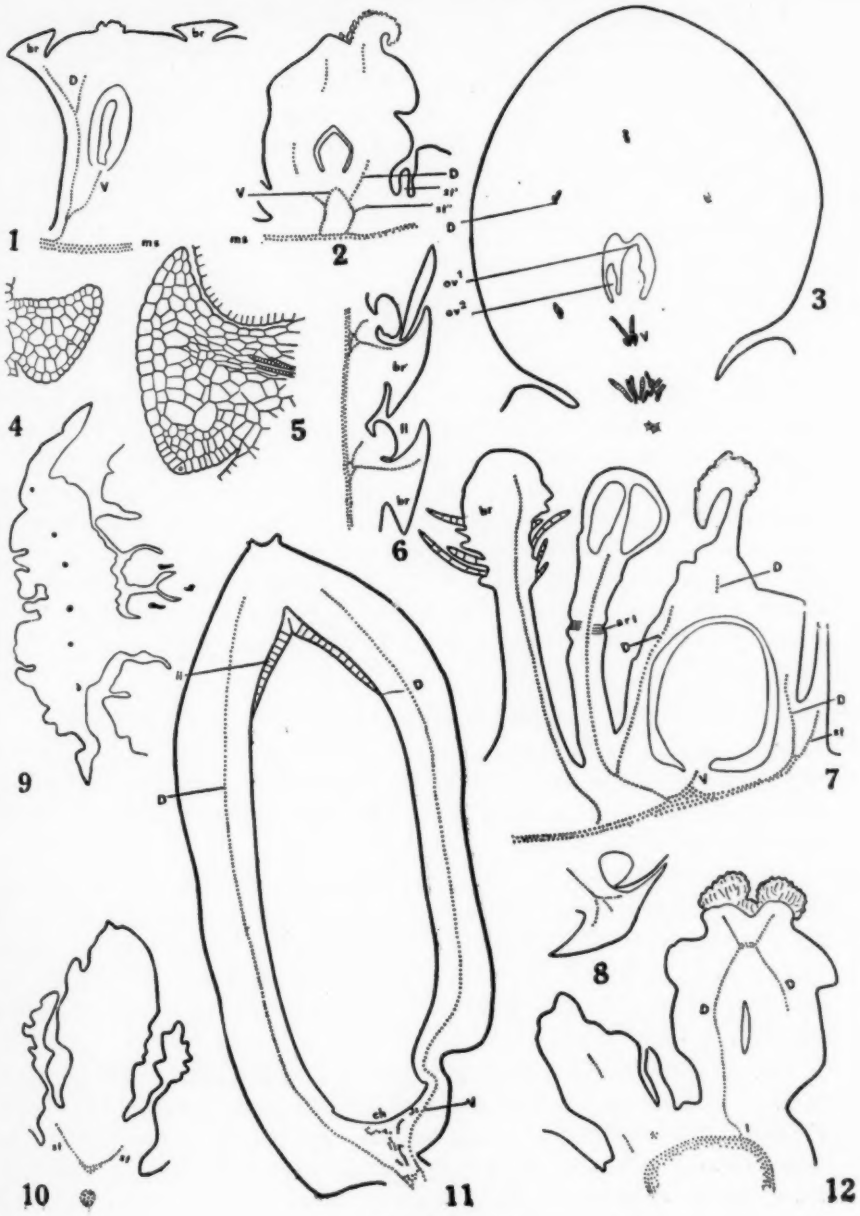
of the inflorescence as a result of the activity of sub-marginal cells (Fig. 4). Some anticlinal divisions of the cells on the lower side bring about elongation of this side of the bract towards the base of the inflorescence also (Fig. 5). Though in earlier stages the upper lobe of the bract appears to be longer (Fig. 6), in older flowers the lower lobe elongates faster and becomes equal to it. In both transverse and longitudinal sections the bract of *P. Gaudichaudianum* looks like a club reaching the height of the anther and stigmas (Fig. 7). Oil cells occur in the mesophyll while stomata are found in the middle of the disc (shield); and hydathodes and multicellular hairs (trichomes) on the surface of the bract.

VASCULAR ANATOMY

The Inflorescence.—The structure of the inflorescence axis in male, female and hermaphrodite spikes is just the same. It consists of two rings of collateral and open bundles, an outer of 10 (*P. betle*) or more (*P. Schmidtii*, *P. Gaudichaudianum*, *P. nigrum*, etc.) and an inner of about five (*P. longum*, *P. betle*, *P. nigrum*, *P. unguiculatum*, etc.). While those of the outer ring are embedded in thick-walled tissue (*P. Gaudichaudianum*, *P. Wightii*, *P. Tucumanum* and *P. obtusilimbum*) a bundle sheath is seen round the bundles of the inner ring in *P. Gaudichaudianum*.

The Flower.—Vasculature of the flower in the different species studied is essentially similar. Whatever differences have been observed are due to variations in the number of stamens, presence or absence of pistil or pistillode and occasionally also due to the fusion of bract or stamens or both with the ovary wall. Hence, the floral supply in the bisexual flower is described in detail and attention will be drawn to the differences in other flowers whenever necessary.

A single branch diverges out somewhat obliquely or horizontally from the parent stele of the inflorescence axis and branches somewhere in the middle of the cortex into a bract and floral supply. In some of the male flowers of *P. Schmidtii*, it was observed that these branches separate off very close to the point of their origin from the parent stele (Fig. 6). However, in the female flowers of *P. longum*, which show complete fusion between bracts and axillant flowers and also between adjacent flowers, the bract trace appears to remain adnate to one of the abaxial bundles in the ovary wall and separates from it only at the level of the micropyle (Fig. 1). Generally the bract trace after bending slightly enters the organ and passes through the upper half of its stalk (Fig. 7). Within the disc (shield of the bract) it divides into two or more (Figs. 8, 9) branches.



TEXT-FIGS. 1–12

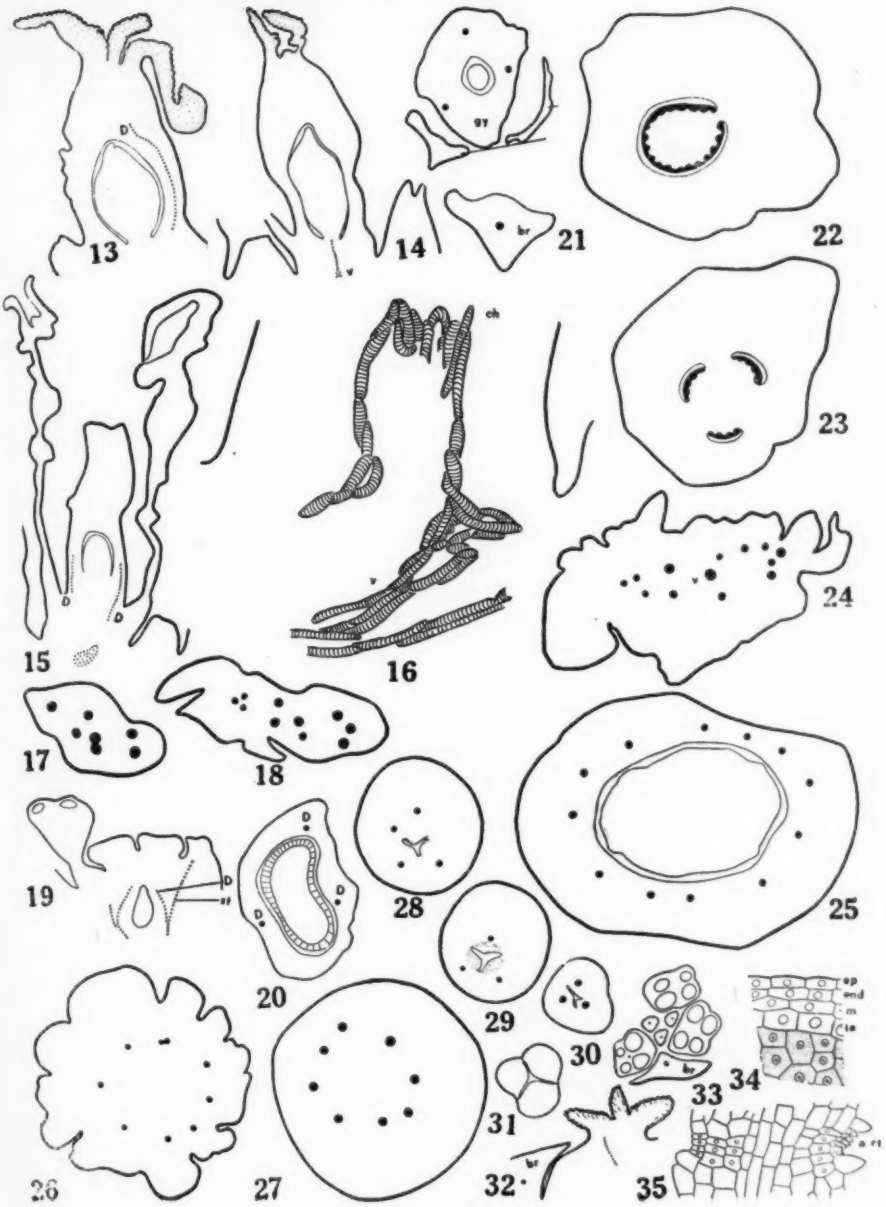
The floral supply reaches the base of the flower where it gives out laterally a trace each to the two stamens [*P. Schmidtii*, *P. betle*, *P. subrubrispicum* (Fig. 10), *P. unguiculatum*, *P. Gaudichaudianum*] and sometimes as in the case of female flowers to two (*P. Wightii*, *P. nigrum*) or three (*P. nigrescens*) staminodes, and then reaches the base of the ovary to supply the gynæceum. The trace that enters the stamen or staminode remains undivided throughout and reaches the connective (Fig. 7). However, in those female flowers (*P. Schmidtii*, Fig. 11) where there are no staminodes the floral supply reaches directly the base of the ovary. Generally in *P. Tucumanum* and, sometimes, in *P. Gaudichaudianum* (Fig. 7) where a certain degree of fusion between the stamens and ovary wall is seen, the stamen traces separate only at a higher level.

The supply to the gynæceum divides at its base into four branches of which one is central and the other three are peripheral as in *P.* sp., *P. Gaudichaudianum* (Fig. 7), *P. longum* (Fig. 12), and *P. subrubrispicum* (Figs. 13, 14 15). The central bundle reaches the chalaza and is more or less completely used up in furnishing a single ovular trace (Fig. 14). This bundle forms a cup of tracheids at the base of the chalaza in *P. Wightii* and *P. Schmidtii* (Fig. 11). The tracheids making up this branch were in one instance seen negotiating a bend (Fig. 16), somewhat similar to that in *Peperomia incana* and *Peperomia comarapana* (Murty, 1958). But in *P. Schmidtii* the central bundle gets separated from one of the peripherals (Figs. 17, 18).

The three peripheral bundles which may or may not branch supply the ovary wall. But in those species where the stamens are adnate to the ovary wall for some distance as in *P. Gaudichaudianum* and *P. Tucumanum* they are compound bundles, *i.e.*, the outer one supplies a stamen while the inner, the ovary wall (Figs. 7, 19).

Except in *P. Schmidtii*, *P. Wightii* and *P. nigrum* the three bundles passing through the ovary wall do not show any branching (Figs. 20, 21) and reach the stigmas where they disappear. They are placed equidistantly. However in *P. longum* these three bundles fuse forming a sort of plate of tracheids from which three small bundles are given off one for each of the three stigmas (Figs. 12, 22, 23).

In the three species mentioned above, *e.g.*, *P. Schmidtii*, *P. Wightii* and *P. nigrum* all the three peripheral bundles divide further so as to give rise to about a dozen bundles (13 in *P. Schmidtii*). As was described earlier one of these in *P. Schmidtii* occupies a central position (Figs. 17, 18) and it supplies the chalaza. Thus in all 9–12 bundles are observed to be arranged in the ovary wall almost uniformly about the level of the chalaza (Figs. 24, 25).



TEXT-FIGS. 13–35

These bundles are placed almost equidistantly from one another. Their number decreases gradually towards the apex of the ovary and only three of them are seen to persist below the level of the stigmas (Figs. 26–30). These continue into the sessile stigmas which are separated by a triradiate stylar canal that appears between them (Figs. 29–32).

The vascular supply in the pistillodes of the male flower, though rudimentary, confirms to the pattern given above (Fig. 33).

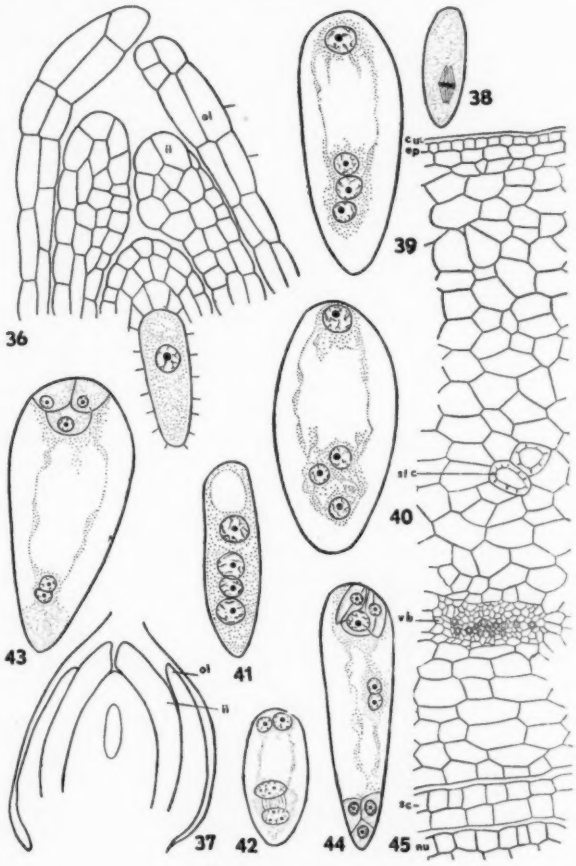
The Microsporangium and the Male Gametophyte.—The development of the microsporangium has been studied in *P. Schmidtii*, *P. Gaudichaudianum*, *P. longum* and *P. unguiculatum*. The anther has four microsporangia (Fig. 33) and a wall consisting of four layers (Fig. 34). The innermost layer, tepetum, remains uninucleate. The sporogenous cells take up a deep stain and develop into two-celled pollen grains. At maturity endothecium becomes fibrous and the middle layer is completely crushed.

It is very interesting to note that the stamens of the *P. Gaudichaudianum*, *P. Tucumanum* and *P. unguiculatum* show a circular constriction about $\frac{2}{3}$ the height of the filament. About 3–4 layers of cells at this region appear to be meristematic (Fig. 35). The constriction becomes more prominent as the stamen matures and the filament breaks off at this point. This special mechanism of separation of the anther may probably have some significance in the method of shedding of the pollen. It has also been used as a character in delimiting the species of *Piper*.

The Megasporangium and the Female Gametophyte.—Though the female gametophyte in *P. Gaudichaudianum* alone could be worked out, some stages observed in *P. Tucumanum* have also been recorded. The tricarpellary gynæceum is unilocular with a single bitegmic orthotropous ovule at the base. The outer integument is 2-layered while the inner is 3-layered below and 4-layered at the apex. Though in earlier stages the outer integument is longer than the inner (Fig. 36), finally the two become equal and the micropyle is formed by the inner integument only (Fig. 37).

A megaspore mother cell is formed as a result of a periclinal division of the primary archesporial cell. Even before the nucleus of the megaspore mother cell undergoes the first division the parietal tissue becomes 2–3-layered in thickness (Fig. 36), and at the 8-nucleate condition it becomes 4-layered in *P. Gaudichaudianum*. In *P. Tucumanum* it is, however, 5-layered at the primary 4-nucleate stage. The megaspore mother cell is much longer than broad (Fig. 36). Its nucleus is centrally situated though it moves to the chalazal end just before it divides (Fig. 38). No vacuole is formed

at this stage and there is no cell-plate formation either. After the second meiotic division all the four nuclei are somewhat towards the chalazal end at the beginning but soon the upper nucleus moves towards the micropylar end, while the other three remain in a linear row at the lower end (Fig. 39) being separated from the upper nucleus by a large vacuole (Fig. 40). A linear row of four nuclei was also observed in *P. Tucumanum* (Fig. 41).



TEXT-FIGS. 36–45

A third division of the nuclei appears to result in a secondary 4-nucleate stage (Fig. 42). Although this particular embryo-sac is very small, the triploid nature of its chalazal nuclei is apparent. Besides, the 1 + 3 arrangement at the primary 4-nucleate stage cannot be reasonably

explained in any other way. It is significant to note that the divisions of these nuclei are seldom simultaneous. In some cases it is the nucleus at the micropylar end that divides first while in others those in the chalazal end may have the lead. One more division in all the four nuclei brings about an 8-nucleate embryo-sac which is broadest on the micropylar side (Fig. 43). The two polars meet each other either in the middle of the embryo-sac (Fig. 44) or towards the chalazal end (Fig. 43). The egg apparatus and the antipodal cells are organized in the usual manner (Figs. 43, 44). The development of the embryo-sac in this species, therefore, follows the *Fritillaria*-type.

The ovary wall at the 8-nucleate stage is about 10 cells thick and is not differentiated into definite regions. Hydathodes and oil cells occur abundantly.

The Fruit Wall.—This has been studied in *P. Schmidtii*, *P. Wightii* and *P. nigrum*. Cooper (1909) described the fruit wall of *P. nigrum*. In all of them the fruit wall is thick and many layered. This is differentiated into three regions. The outer one is fleshy and several layered and is bounded on outside by a layer of epidermis. The epidermal cells have thickened outer walls covered by a thick layer of cuticle. Inner to the epidermis there are 1–3 layers of hypodermis where cells are small and somewhat thick-walled. In *P. nigrum* these cells are elongated at right angles to the surface and often show a narrow central lumen. Next to the hypodermis there are 14–20 layers of parenchyma in which are scattered groups of stone cells (Fig. 45). The second region consists of 3–4 layers of small cells and the vascular bundles traverse through this region. The third one has about 6–8 layers of cells. In *P. Schmidtii* only parenchyma cells occur while in *P. Wightii* it contains thick-walled cells that are somewhat elongated parallel to the surface. This region contains oil cells scattered here and there.

The seed-coat shows 2 (*P. nigrum*) or 3 (*P. Wightii*) layers. The outer one is completely crushed and in its place there remains a dark layer without any differentiation of its cells. The perisperm cells are very prominent with numerous starch grains in the vacuoles.

DISCUSSION AND CONCLUSIONS

The course of the vascular supply to the bract and its axillant flower is fundamentally same in all the flowers studied. From the position and course, the three peripheral bundles at the base of the ovary are to be interpreted as the carpellary dorsals and the one that supplies the chalaza as the ventral (Placental) strand which is consumed entirely in supplying the single ovular trace. Additional bundles observed in the ovary wall of *P. Schmidtii*,

P. nigrum and *P. Wightii* appear to constitute secondary laterals. However, such a typical plan of vascular construction is modified as a result of adnation of bract or stamens.

In the female flower of *P. longum* not only the bract is fused to the ovary wall for quite a long distance, even its bundle shows fusion with one of the carpellary bundles running on the abaxial side, separating only at about the level of the micropyle. Such a fusion of the bract with its axillant flower has been reported in *Juglans* (Manning, 1940), *Lonicera* (Wilkinson, 1944) and especially in the genera showing inferior ovaries. Occasionally in *P. Gaudichaudianum* and generally in *P. Tucumanum* where the stamens arise somewhat above the base of the ovary and at the level of the stigmas respectively the carpellary dorsals and the stamen traces separate only at this level. Such an adnation of the stamen and carpellary traces (or bundles ?) has also been observed by the author in *Houttuynia* (Murty, 1956) and in *Chloranthus* (Swamy and Bailey, 1950). As the flowers are naked, it is difficult to say whether such a condition should be described as semi-inferior (*P. Gaudichaudianum*) and inferior (*P. Tucumanum*).

The Number and Position of Carpels.—On the basis of the number of stigmas the *Piper* ovary is generally interpreted as 2–5 carpellary (Rendle, 1938, Lawrence, 1951). Baillon (1874), however, interpreted it as monocarpellary and the three stigmas as three lobes but at the same time he admitted that occasionally there may be flowers with two or more carpels. Eckardt (1937) also discussed at some length the pseudomonomerous condition of the ovary.

As far as the author is concerned there is little doubt that the *Piper* gynæceum consists of three carpels. This is supported by the fact that there are, in the ovary wall, three dorsal bundles which continue either directly or after forming a ring (*P. longum*) into the three stigmas. There is no justification, whatsoever, to interpret the three stigmas as merely lobes of a single one. They are definitely three stigmas representing three carpels.

Joshi (1944) described the gynæceum of *P. longum* to be bicarpellary as he observed four bundles in the ovary wall; two of which he interpreted as dorsals and the other two as fused ventrals. The present observations on the same species did not reveal such a condition. It appears, that Joshi mistook the bract bundle which separates from one of the dorsals at a higher level as a carpellary bundle. Actually there are only three dorsals which traverse the ovary wall. The placental strand formed by the fusion of the ventrals is consumed more or less completely in furnishing a single ovular trace. The

three stigmas at the top of the ovary too are quite evident (Fig. 32). Hence in *P. longum* also there are three carpels.

Not only all the species studied agree in the number of carpels, but they also show the same pattern in their arrangement. Out of the three dorsals two are always found to be antero-oblique and the third posterior. The stigmas which are carinal also occupy the same positions. The solitary ovule apparently belongs to one of the carpels.

The Placentation.—The solitary ovule is often described as basal. Baillon (1874) described it as sub-basillar. In *Peperomia* some anatomical evidence has been brought forward to show that the single apparently basal ovule has been derived from lateral position (Murty, 1952). Although no such evidence exists in *Piper* there is little doubt that the condition here too is the same as in *Peperomia* and that the basal position of the ovule is a derived one. The formation of the ventral strand from the carpellary dorsals in *P. Schmidtii*, the presence of a bend in one instance in the course of the ventral strand of *P. longum* seem to confirm such a belief. A rudimentary second ovule like the one reported in *Peperomia Peirescifolia* (Murty, 1956, 1958) has been observed in *P. Wightii* but it will not be advisable to speculate on this count.

The Megasporangium and the Female Gametophyte.—Unlike the condition in *Peperomia*, the *Piper* anthers are four-locular and the ovules are bitegmic. Generally as has been described by Johnson (1910) and Joshi (1944) the micropyle is formed by the inner integument alone though Johnson (1902) described and figured a much larger outer integument in *P. medium*. Such a difference in closely related species is according to Joshi (1944) unlikely. The difference may probably be due to a difference in the stages of the ovules examined. As was described earlier the condition in a young ovule is different from that in a mature one.

The 1 + 3 arrangement of the megaspore nuclei, the presence of three nucleoli in the chalazal nuclei at the secondary 4-nucleate stage and then the subsequent divisions and the ultimate formation of 8-nucleate embryo-sac showing an egg apparatus with 2 synergids, 2 polars and three antipodals observed in *P. Gaudichaudianum* lead to the conclusion that the embryo-sac in *Piper* belongs to the *Fritillaria*-type and not to the *Adoxa*-type as was believed by Johnson (1902, 1910), Fisher (1914), etc. This conclusion is in agreement with that of Swamy (1944) and Joshi (1944) regarding *P. betel* and *P. longum* respectively.

Summary

The vascular anatomy of the flower in general is uniform in all the species of *Piper* studied. A flower is always supplied by a single branch that gives out first a trace to the bract. The remaining vascular supply gives out traces to the stamens or staminodes when present and then supplies the gynæceum. Only in some species like *P. Tucumanum* adnation of the stamen traces and in the female flowers of *P. longum* of the bract trace with the carpellary dorsals, has been observed. The gynæceum supply splits up at its base into three dorsals and one ventral strand. Secondary laterals are observed only in three species. In *P. longum* the dorsals are seen fusing below the stigmas otherwise they directly supply them.

Embryology in *P. Gaudichaudianum* has been studied and it is concluded that it follows *Fritillaria*-type as in other species of *Piper*.

Acknowledgements

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EXPLANATION OF TEXT-FIGURES

FIGS. 1–12. Fig. 1. Part of a longitudinal section of female spike of *P. longum* showing adnation of bract to the ovary, and bract trace with the carpellary dorsal, × 13. Figs. 2 and 3. Two flowers of *P. Wightii* cut longitudinally. Note the staminode and its trace in Fig. 2 and an extra ovule in Fig. 3, × 13. Figs. 4 and 5. *P. Schmidtii* showing stages in the development of bract, × 133. Fig. 6. Part of longitudinal section of a young male spike of *P. Schmidtii* showing the arrangement of bracts and vascular supply to the flower and bract, × 13. Fig. 7. Semi-diagrammatic longitudinal section of a flower of *P. Gaudichaudianum* showing vascular supply to bract and different organs of flower. Note the articulation on the filament and adnation of stamen and carpellary dorsal for some distance, × 53. Fig. 8. A bract of *P. Schmidtii* showing the branching of the bract bundle in the shield of the bract, × 13. Fig. 9. Bract of *P. nigrum* showing many bundles within its shield, × 30. Fig. 10. Flower of *P. subrubrispicum* in longitudinal section showing vascular supply to the two stamens, × 133. Fig. 11. Female flower of *P. Schmidtii* in longitudinal section showing the ventral strand and carpellary dorsals. Fig. 12. Cross-section of a spike of *P. longum* showing the course of two carpellary dorsals, × 30.

art., articulation; *br.*, bract; *ch.*, chalaza; D., carpellary dorsal; *fl.*, flower; *ii.*, inner integument; *ms.*, main vascular supply of the inflorescence axis; ov^1 and ov^2., fertile and rudimentary ovules; *st.*, stamen trace; *St'.*, staminode; *St".*, staminode trace; *V.*, ventral strand.

FIGS. 13–35. Figs. 13–15. Flowers of *P. subrubrispicum* in longitudinal sections showing vascular supply to the ovary, × 133. Fig. 16. A well-developed ventral strand in *P. longum* showing a bend in its course, × 53. Figs. 17 and 18. Cross-sections of female flower of *P. Schmidtii* showing the formation of ventral strand, carpellary wall bundles, × 30. Fig. 19. Longitudinal section of a flower of *P. Tucumanum* showing the vascular supply to the stamens. Note the adnation of stamen traces and carpellary dorsals for some distance, × 30. Fig. 20. Cross-section of an ovary of *P. Gaudichaudianum* showing the three carpellary dorsals, × 13. Fig. 21. Cross-section of a flower of *P. subrubrispicum* showing bract, two stamens and gynæceum, × 58. Figs. 22 and 23. Cross-sections of an ovary of *P. longum* showing the formation of a ring of vascular tissue below the stigma region (Fig. 22) and its division into

bundles (Fig. 23) that supply the three stigmas, × 30. Figs. 24 and 25. Cross-sections of ovary of *P. Schmidtii* and *P. nigrum* respectively showing 12 wall bundles. Note the ventral strand in Fig. 24, × 30. Fig. 26. *P. Wightii* and Figs. 27–29. *P. Schmidtii*. Cross-sections of ovary showing reduction in the number of wall bundles to three towards the apex. Fig. 26, × 13. Figs. 27–29, ×30. Figs. 30 and 31. Cross-sections of the apex of the ovary of *P. nigrum*, showing the formation of stigmas and the disappearance of traces, × 30. Fig. 32. Apex of the ovary of *P. longum* showing the three stigmas, × 30. Fig. 33. Cross-section of male flower of *P. longum* showing the position of bract, three stamens and three stigmas, × 30. Fig. 34. A part of cross-section of anther of *P. Schmidtii* showing the wall layers and sporogenous cells, × 533. Fig. 35. Shows the arrangement of cells at the place of articulation on the filament of *P. Gaudichaudianum*, × 53.

art., articulation; *br.*, bract; *ch.*, chalaza; D., carpellary dorsal; *end.*, endothecium; *ep.*, epidermis; *gy.*, gynæceum; *m.*, middle layer; *St.*, stamen trace; *ta.*, tapetum; V., ventral strand.

Figs. 36–45. Figs. 36, 38–40, 42–44. *P. Gaudichaudianum* and Figs. 37 and 41. *P. Tucumanum*. Fig. 36. Upper part of a young ovule showing the two integuments, megaspore mother cell and parietal tissue, × 533. Fig. 37. Ovule with two integuments. Note the inner integument is longer, ×133. Fig. 38. Megaspore mother cell with its nucleus undergoing division, ×567. Figs. 39 and 41. Show the arrangement of nuclei at the primary 4-nucleate stage of the embryo-sac. Figs. 39 and 40, × 567; Fig. 41, × 533. Fig. 42. Embryo-sac showing secondary 4-nucleate condition. Note the number of nucleoli in the two nuclei on the chalazal side, × 567. Fig. 43. Embryo-sac with egg apparatus and two polar nuclei, × 567. Fig. 44. 8-nucleate embryo-sac, × 567. Fig. 45. Part of a cross-section of fruit wall of *P. Wightii*, × 133.

Cu., cuticle; *ep.*, epidermis; *ii.*, inner integument; *nu.*, nucellus; *Oi.*, outer integument *Sc.*, seed-coat; *Stc.*, stone cell; *Vb.*, vascular bundle.

THE ALGAL FLORA OF VINDHYAN FORMATIONS OF THE MIRZAPUR DISTRICT, U.P.*

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Although a number of contributions[1-4] have been made from this department on the Myxophyceæ of the United Provinces (Uttar Pradesh), but none of these have considered the algal flora of the Vindhyan Formations of this State. The present study, therefore, was planned to fill up this important gap.

The collections were made from Motia Tal, Astbhuji and Windhyam Falls in October 1947 in connection with a botanical excursion to that area led by Dr. R. N. Singh. In all forty-two forms have been described and out of these thirty-seven are blue-greens and five belong to Chlorophyceæ. The habitat of these plants is varied; ten have been collected growing on soil, seventeen from moist stones and rocks, and fifteen from tanks, ponds and other aquatic situations.

Systematic Enumeration of the Species Observed

Myxophyceæ

1. *Aphanocapsa grevillei* (Hass.) Rabenh. Geitler, in Rabenhorst's *Kryptogamen-Flora von Europa*, Band XIV, *Cyanophyceæ*, 1930–32, p. 158, Fig. 71.

Lat. cell., 3·5–4·5 μ.

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[2] Rao, C. B., "The Myxophyceæ of the United Provinces, India—II," *Ibid.*, 1936, **3 B** (2).

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Habitat.—On the bank of Motia Tal along with *Lyngbya* species.

2. *Aphanocapsa pulchra* (Kütz) Rabenh. Geitler, *op. cit.*, 1930–32, p. 156, Fig. 69 *g*.

Lat. cell., 3·2–4·8 μ.

Habitat.—On moist rock along with *Oscillatoria* species, Windhyam Falls.

3. *Microcystis æruginosa* Kütz. Geitler, *op. cit.*, 1930–32, p. 136, Fig. 59 *d*.

Lat. cell., 2·5–3·5 μ.

Habitat.—In a small puddle along with *Chroococcus montanus*, Windhyam Falls.

4. *Chroococcus montanus* Hansg. Geitler, *op. cit.*, 1930–32, p. 236.

Lat. colon. *cum* vag., 15·3–17·8 μ; lat. cell., 5·2–6·0 μ; long. cell., 4·3–5·2 μ.

Habitat.—In a small puddle along with *Microcystis æruginosa*, Windhyam Falls.

5. *Synechococcus cedrorum* Sauv. Geitler, *op. cit.*, 1930–32, p. 273.

Lat. cell., 5–6·5 μ; long. cell., 6·8–8·5 μ.

Habitat.—On moist rock and soil along with *Phormidium* species, Windhyam Falls.

6. *Oscillatoria subbrevis* Schmidle. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *b*.

Lat. trich., 4·3–5·2 μ; long. cell., 1·5–2·0 μ.

Habitat.—On the bank of Motia Tal along with *Oscillatoria princeps* and *Phormidium angustissimum*; Motia Tal.

7. *Oscillatoria princeps* Vauch. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *c–g*.

Lat. cell., 23·8–34·0 μ; long. cell., 3·5–5·7 μ.

Habitat.—On the bank of Motia Tal along with *Oscillatoria subbrevis* and *Phormidium angustissimum*, Motia Tal.

8. *Oscillatoria formosa* Bory. Geitler, *op. cit.*, 1930–32, p. 971, Fig. 619 *b*.

Lat. trich., 3·6–5·4 μ; long cell., 2·4–4·8 μ.

Habitat.—On moist soil along with *Oscillatroia splendida*, Windhyam Falls.

9. *Oscillatoria splendida* Grev. Geitler, *op. cit.*, 1930–32, p. 973, Fig. 620 *d–f.*

Lat. trich., 3·0–4·5 μ; long. cell., 4·5–7·8 μ.

Habitat.—On moist soil along with *Oscillatroia formosa*, Windhyam Falls.

10. *Phormidium angustissimum* W. *et* G. S. West. Geitler, *op. cit.*, 1930–32, p. 996.

Lat. cell., 1·0–1·2 μ; long. cell., 3·5–4·5 μ.

Habitat.—On moist soil along with *Oscillatoria princeps* and *Oscillatoria subbrevis*, Motia Tal.

11. *Phormidium purpurascens* (Kütz) Gom. Geitler, *op. cit.*, 1930–32, p. 1007, Fig. 644 *c.*

Lat. fil., 3·5 μ; lat. trich., 1·7–2·5 μ; long. cell., 1·7–5·2 μ.

Habitat.—On the steps of Motia Tal along with *Calothrix fusca* and *Lyngbya rubida.*

12. *Phormidium corium* (Ag.) Gom. Geitler, *op. cit.*, 1930–32, p. 1017, Fig. 649 *c.*

Lat. fil., 2·5–3·5 μ; long. cell., 3·5–5·2 μ.

Habitat.—On moist soil along with *Tolypothrix distorta*, Windhyam Falls.

13. *Phormidium inundatum* Kütz. Geitler, *op. cit.*, 1930–32, p. 1017, Fig. 649 *e.*

Lat. trich., 4·5–5·0 μ; long. cell., 6·3–9·0 μ.

Habitat.—On moist rock along with *Scytonema saleyeriensis* var. *indica* Bhâradwâja, Windhyam Falls.

14. *Phormidium ambiguum* Gom. Geilter, *op. cit.*, 1930–32, p. 1015, Fig. 647 *e.*

Lat. fil., 9·9–10·8 μ; lat. trich., 7·2–8·1 μ; long. cell., 2·0–2·5 μ.

Habitat.—On moist rock, Windhyam Falls.

15. *Phormidium cebennense* Gom. Frémy, Les Myxophycées de l' Afrique équatoriale francaise, *Arch. Bot.*, 3, Mém, 2, 1930, p. 147, Fig. 129.

Lat. trich., 1·8–2·0 μ; long. cell., 3·0–5·4 μ.

Habitat.—On moist rock, Windhyam Falls.

16. *Lyngbya kützingii* Schmidle. Geitler, *op. cit.*, 1930–32, p. 1036, Fig. 654 *a*.

Lat. fil., 3·0–3·5 μ; long. cell., 0·8–1·7 μ.

Habitat.—Epiphytic on *Oedogonium* filaments, Windhyam Falls.

17. *Lyngbya æstuarii* Liebm. Geitler, *op. cit.*, 1930–32, p. 1052, Fig. 666.

Lat. fil., 23·1–24·7 μ; lat. trich., 13·2–15·0 μ; crass. vag., 4·9–5·7 μ; long. cell., 4·5–6·0 μ.

Habitat.—On moist rock, Windhyam Falls.

18. *Lyngbya major* Menegh. Geitler, *op. cit.*, 1930–32, p. 1066, Fig. 679 A.

Lat. fil., 20·8–23·3 μ; lat. trich., 15·7–17·4 μ; crass. vag., 2·5–3·0 μ; long. cell., 3·5–8·7 μ.

Habitat.—On moist rock along with *Lyngbya truncicola*, Motia Tal.

19. *Lyngbya truncicola* Ghose. Ghose, " A systematic and ecological account of the blue-green algæ of Lahore and Simla," *Jour. Linn. Soc. Bot.*, 1923, Pl. 31, Fig. 6.

Lat. fil., 11·9–13·0 μ; lat. trich., 8·5–10·4 μ; long. cell., 1·9–3·5 μ.

Habitat.—On moist rock along with *Lyngbya major*, Motia Tal.

20. *Lyngbya rubida* Frémy. Geitler, *op. cit.*, 1930–32, p. 1055, Fig. 668 *c*.

Lat. fil., 6·4–8·2 μ; tat. trich., 4·6–5·6 μ; long. cell., 3·2–6·4 μ.

Habitat.—On moist soil, Windhyam Falls.

21. *Lyngbya confervoides* Ag. Geitler, *op. cit.*, 1930–32, p. 1061, Fig. 672 *b*.

Lat. fil., 20·8–23·3 μ; lat. trich., 15·7–17·4 μ; long. cell., 3·5–8·7 μ; crass. vag., 2·5–3·0 μ.

Habitat.—On moist rock, Motia Tal.

22. *Lyngbya ærugineo-cærulea* (Kütz) Gom. Frémy, *loc. cit.*, 1930, p. 193, Fig. 157.

Lat. fil., 6·4–8·0 μ; lat. trich., 5·1–6·4 μ; long. cell., 1·4–4·8 μ.

Habitat.—In a puddle, Motia Tal.

23. *Microcoleus chthonoplastes* Thuret. Geitler, *op. cit.*, 1930–32, p. 1134, Fig. 739.

Lat. cell., 3·8–4·9 μ; long. cell., 3·8–5·7 μ.

Habitat.—On moist rock, Windhyam Falls.

24. *Anabæna unispora* Gardner. Geitler, *op. cit.*, 1930–32, p. 902, Fig. 580 *b*.

Long. trich., 450–700 μ; lat. cell., 3·6–5·7 μ; long. cell., 3·5–11·2 μ; lat. het., 5·2–8·2 μ; long. het., 6·8–12·1 μ; lat. spore., 6·8–12·1 μ; long. spore., 18·9–24·8 μ.

Habitat.—On moist soil along with *Aulosira fertilissima*, Motia Tal.

25. *Nostoc spongiæforme* Ag. Geitler, *op. cit.*, 1930–32, p. 840, Fig. 531.

Lat. cell., 3·5–4·5 μ; long. cell., 3·6–6·9 μ; lat. het., 5·2–6·2 μ; long. het., 5·2–6·8 μ; lat. spore., 6·2–7·5 μ; long. spore., 10·2–12·2 μ.

Habitat.—On moist soil along with other algæ, Windhyam Falls.

26. *Aulosira fertilissima* Ghose. Ghose, *loc. cit.*, 1923, p. 342, Fig. 9.

Lat. fil., 6·5–10·5 μ; long. cell., 5·5–18·8 μ; lat. het., 4·5–7·5 μ; long. het., 8·2–15·5 μ; lat. spore., 6·2–8·8 μ; long. spore., 10·5–19·2 μ.

Habitat.—On moist soil along with *Anabæna unispora*, Motia Tal.

27. *Cylindrospermum muscicola* Kütz. Geitler, *op. cit.*, 1930–32, p. 822.

Lat. trich., 3·5–5·7 μ; long. cell., 5·2–11·2 μ; lat. het., 5·1–8·1 μ; long. het., 10·1–12·1 μ; lat. spore., 9·0–12·1 μ; long. spore., 10·1–20·1 μ.

Habitat.—On moist soil along with other algæ, Motia Tal.

28. *Calothrix braunii* Born. *et* Flah. Geitler, *op. cit.*, 1930–32, p. 606, Fig. 381.

Lat. fil., 9·0–10·0 μ; lat. trich., 6·7–7·5 μ; long. cell., 4·5–10·5 μ; lat. het., 6·5–8·0 μ; long. het., 4·5–7·5 μ.

Habitat.—On moist rock, Windhyam Falls.

29. *Calothrix fusca* Born. *et* Flah. Geitler, *op. cit.*, 1930–32, p. 611, Fig. 384.

Lat. fil. at base 15·0–19·5 μ; lat. fil. at top 4·0–6·9 μ ; lat. trich., 7·8–13·9 μ at base; lat. trich. at top 3·5–4·5 μ; long. cell., 1·7–3·5 μ; lat. het., 8·0–10·4 μ; long. het., 5·2–8·7 μ.

Habitat.—On moist rock, Windhyam Falls.

30. *Dichothrix fusca* Fritsch. Geitler, *op. cit.*, 1930–32, p. 593.

Lat. fil., 12·0–14·5 μ; lat. trich., 5·2–8·7 μ; long. cell., 3·5–9·6 μ; lat. het., 10·6–14·0 μ; long. het., 10·6–12·0 μ.

Habitat.—On rock along with colonial blue-green algæ, Windhyam Falls.

31. *Microchæte uberrima* Carter. Geitler, *op. cit.*, 1930–32, p. 669, Fig. 430.

Lat. fil., 16·2–18·0 μ; lat. trich., 9·9–14·4 μ; long. cell., 9·9–16·6 μ; lat. het., 9·9–10·8 μ; long. het., 20·2–32·4 μ; lat. spore., 12·6–14·4 μ; long. spore., 10·8–36·0 μ.

Habitat.—In a puddle with *Tolypothrix distorta*, Windhyam Falls.

32. *Scytonema stuposum* (Kütz) Born. Geitler, *op. cit.*, 1930–32, p. 757, Fig. 482.

Lat. fil., 21·0–26·5 μ; lat. trich., 10·0–15·9 μ; crass. vag., 5·5 μ; long. cell., 3·5–6·9 μ; lat. het., 13·9–17·4 μ; long. het., 6·9–10·6 μ.

Habitat.—On moist rock along with *Calothrix braunii*, Windhyam Falls.

33. *Scytonema saleyeriensis* var. *indica* Bhâradwâja. Bhâradwâja, "The Taxonomy of *Scytonema* and *Tolypothrix*, including some new records and new species from India and Ceylon," *Revue Algologique*, 1933, p. 162, Fig. 2 E.

Lat. fil., 18·0–21·6 μ; lat. trich., 10·8–12·6 μ; long. cell., 2·2–3·6 μ; lat. het., 12·6–14·4 μ; long. het., 9·0–12·6 μ; crass. vag., 4·5–5·0 μ.

Habitat.—On moist rock along with *Microcoleus chthonoplastes* and *Phormidium inundatum*, Windhyam Falls.

34. *Scytonema hofmanni* Ag. Geitler, *op. cit.*, 1930–32, p. 773, Fig. 495.

Lat. fil., 8·7–11·1 μ; lat. trich., 5·2–6·9 μ; long. cell., 3·5–6·9 μ; lat. het., 5·2–6·9 μ; long. het., 8·7–13·9 μ.

Habitat.—On moist rock, Windhyam Falls.

35. *Scytonema pascheri* Bhâradwâja. Bhâradwâja, *loc. cit.*, 1933, p. 159, Fig. 1 *c–d*.

Lat. fil., 17·4–19·1 μ; crass. vag., 3·5 μ; lat. trich., 10·4–12·1 μ; long. cell., 5·2–11·9 μ; lat. het., 12·2–13·8 μ; long. het., 24·4–28·9 μ.

Habitat.—On soil, Motia Tal.

36. *Tolypothrix distorta* Kütz. var. *samænsis* Wille. Geitler, *op. cit.*, 1930–32, p. 721.

Lat. fil., 14·0–19·8 μ; crass. vag., 3·3–4·5 μ; lat. trich., 13·2–14·8 μ; long. cell., 3·3–11·5 μ; lat. het., 13·2–14·8 μ; long. het., 10·0–23·0 μ.

Habitat.—In a small puddle, Motia Tal.

37. *Tolypothrix distorta* Kütz. var. *brevis* Gardner. Geitler, *op. cit.*, 1930–32, p. 719.

Lat. fil., 17·4–18·1 μ; crass. vag., 3·5 μ; lat. trich., 12·9–13·6 μ; long. cell., 6·9–15·3 μ; lat. het., 12·9–13·6 μ; long. het., 13·6–30·6 μ.

Habitat.—On moist soil, Motia Tal.

Chlorophyceæ

38. *Oedogonium mesospirale* Singh. Singh, "The Oedogoniales of the United Provinces, India—I," *Proc. Ind. Acad. Sci.*, 1938, **8** (5), p. 379, Fig. 3 B–E.

Lat. cell. veg. fem., 26·4–33·0 μ; long. cell. veg. fem., 78·5–125·3 μ; lat. cell. veg. masc., 26·4–29·7 μ; long. cell. veg. masc., 72·6–132·5 μ; crass. oog., 52·4–56·2 μ; long. spore., 46·5–52·5 μ; lat. androsp., 26·4–29·7 μ; long. androsp., 8·9–13·2 μ; lat. nannand. stipes., 13·2–16·5 μ; long. nannand. stipes., 30·0–45·9 μ; lat. anth., 9·2–13·2 μ; long. anth., 6·9–9·9 μ.

Habitat.—From a pond along with *Bulbochæte bhâradwâjai*, Astbhuji.

39. *Oedogonium capilliforme* (Kütz.) Wittrock. Tiffany, *Oedoginiaceæ*, Ohio, 1934, p. 127, Pl. XLIX, Fig. 472.

Lat. cell. veg. fem., 40·0–42·2 μ; long. cell. veg. fem., 160·5–211·5 μ; lat. cell. veg. masc., 39·6–43·5 μ; long. cell. veg. masc., 165·5–203·5 μ; crass. oog., 50·5–56·5 μ; long. oog., 66·6–79·6 μ; cross. oosp.,

48·5–50·5 μ; long. oosp., 56·5–60·5 μ; lat. anth., 35·5–39·5 μ; long. anth., 5·5–9·5 μ.

Habitat.—From a pond, Astbhuji.

40. *Oedogonium sociale* Kütz. Tiffany, *op. cit.*, 1934, p. 67, Pl. 11, Fig. 109.

Lat. cell. veg. fem., 14·5–15·9 μ long. cell. veg. fem., 26·4–55·5 μ; lat. cell. veg. masc., 13·2–15·5 μ; long. cell. veg. masc., 25·5–30·5 μ; crass. oog., 33·5–35·5 μ; long. oog., 33·9–35·5 μ; crass. oosp., 31·5–32·2 μ; long. oosp., 29·7–31·0 μ; lat. anth., 11·5–13·0 μ; long. anth., 6·6–8·0 μ.

Habitat.—From a temporary pond, Astbhuji.

41. *Bulbochæte bhâradwâjai* Singh. Singh, *loc. cit.*, 1938, p. 394, Figs. 64–65.

Lat. cell., 12·5–16·5 μ; long. cell., 17·2–18·9 μ ; crass. oog., 27·5–29·7 μ; long. oog., 38·5–41·8 μ; crass. oosp., 23·1–26·4 μ; long. oosp., 32·5–36·3 μ; lat. androsp., 12·5–13·2 μ; long. androsp., 13·5–14·5 μ; lat. nannand. stipes., 11·5–13·2 μ; long. nannand. stipes., 16·7–18·9 μ; lat. anth., 6·6–8·3 μ; long. anth., 4·5–5·5 μ.

Habitat.—Epiphytic on *Oedogonium socale*, Astbhuji .

42. *Spirogyra juergensii* Kütz. Jao, "Studies on the Freshwater Algæ of China, I. Zygnemataceæ from Szechwon," *Sinensia*, 1935, **6** (5), p. 631, Fig. 59.

Lat. veg. cell., 28·0–35·5 μ; long. veg. cell., 45·0–80·0 μ; lat. spore., 27·5–35·0 μ; long. spore., 42·0–64·4 μ.

Habitat.—From a pond along with *Bulbochæte bhâradwâjai*, Astbhuji.

In conclusion, the author expresses his great indebtedness to Prof. Dr. Y. Bhâradwâja, M.SC., PH.D. (London), F.L.S., F.N.I., F.A.SC., and Dr. R. N. Singh, D.SC., for their kind guidance and criticism throughout the course of this study.

AHOLCUS EUPROCTISCIDIS MANI (SCELIONIDÆ: HYMENOPTERA), AN EGG PARASITE OF *EUPROCTIS LUNATA* WALKER

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Received June 13, 1958

The family Scelionidæ comprises a large number of genera of small size insects which have a unique character of parasitising eggs only, particularly those of Lepidoptera, Hemiptera, Orthoptera, Diptera and Arachnida. However, there are instances where they parasitise the eggs of coleopterous and neuropterous insects as well. Representatives of this family have been successfully employed in the biological control of insect pests in Hawaii, New South Wales and other regions. It is rather surprising that the biology and morphology of the immature stages of this group are not clearly known, though some information is available here and there. In this country the biology of the Scelionidæ has not been worked out before. This family exhibits some peculiarities in the morphology and in the development of the tracheal system in the immature stages. These aspects have been studied in detail in this investigation of the biology and morphology of the Scelionid.

Habits of the Adult Parasite

Aholcus euproctiscidis Mani is an important egg parasite of *Euproctis lunata* Walker that is a serious pest of castor throughout India. The damage caused by the voraciously feeding caterpillars is sometimes colossal and instances are not rare when the whole crop has been destroyed. The adult parasites are seen visiting the castor leaves in search of their host eggs during August, September and October when the host population is at its height.

Pre-oviposition Period

The parasites mate soon after emergence and the pre-ovipositional period varies from 12 to 24 hours.

Oviposition

The female parasite when it comes in the vicinity of the host egg mass examines it by gentle tapping of its antennæ and makes its way to the eggs through the fluffy fibrous material covering them. It then inserts the

ovipositor through the egg shell. Laboratory studies and field observations have indicated that the time taken to lay an egg varies from 5 to 10 minutes or more at times.

As a rule only one egg is deposited in a host egg. However, observations have shown that sometimes two eggs are laid in a single host egg. However, in every such case only one individual successfully completed its development and emerged as an adult, even though more than one egg was laid in the host egg.

Immature Stages

The Egg (Fig. 1, 1).—The egg is stalked, the body being spindle-shaped and measures 0·095 mm. in length. The pedicel measures 0·056 mm. in length while the spindle-shaped body is 0·044 mm. wide. The egg is translucent and is seen floating in the yolk of the host egg.

First Instar Larva (Fig. 1, 2).—The first instar larva of Scelionidæ is called the " teleaform " larva. It hatches out after an incubation period of 1 to 2 days. Its body is unsegmented, but is divided by a sharp constriction into two regions, namely, a cephalothorax and an abdomen, both being curved dorsally. At the cephalic end of the body there are two curved and pointed mandibles. On the ventral side of the thoracic region there is a small process that disappears in the second instar larva. The mandibles are long and fleshy and unsclerotised. There is a band of setæ separating the cephalothorax and the abdomen. The abdomen is globular and ends in two equally developed caudo-ventral horns that are unsclerotised and directed forwards.

As the first instar larva develops its size increases. A sac-like yellow alimentary tract appears within the abdomen and one of the caudo-ventral horns becomes reduced in length. When the larva is 2 days old, two more bands of setæ appear below the first band, one towards the ventral side and the other towards the dorsal side (Fig. 1, 3). As further development takes place the sac-like alimentary tract becomes larger.

The maximum length of this stage was 0·37 mm. and width 0·27 mm.

Second Instar Larva (Fig. 1, 4).—After 2 to 3 days of feeding and development, the larva moults. The second instar larva is irregularly ovoid. The mandibles are still well developed, but the setæ become irregular in arrangement. The caudo-ventral horns become reduced; one becomes much shorter than the other. The larva also becomes reduced in size. There is no trace of body segmentation or tracheal system or spiracles. The duration of the second stage is very short and lasts for about a day.

The maximum length of the second instar larva was 0·34 mm. and width 0·25 mm.

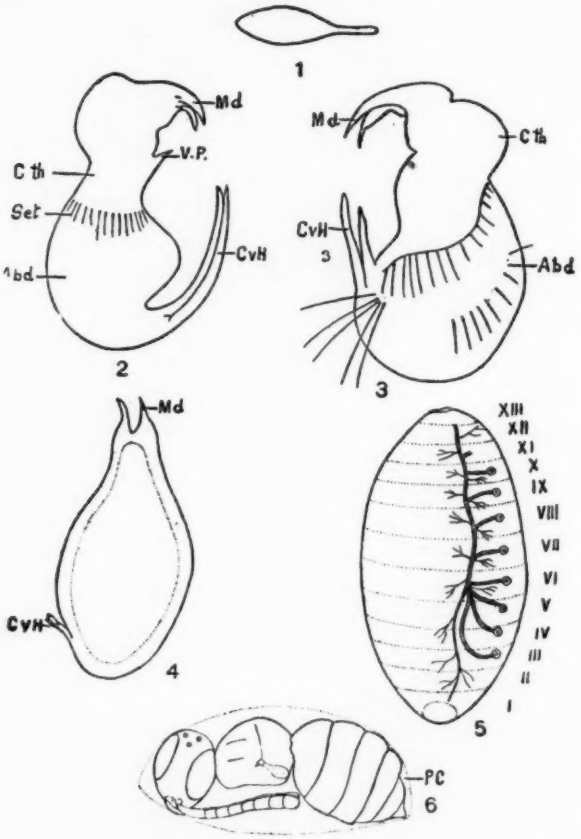

FIG. 1.—1. Egg. 2. Early first instar. 3. Late first instar. 4. Second instar larva 5. Arrangement of tracheal system in the third instar larva. 6. Pupa.—*Abd*-Abdomen; *Cth*-Cephalothorax; *CvH*-Caudoventral horn; *Md*-Mandible; *Set*-Setæ; *V.P.*-Ventral Process.

Third Instar Larva (Fig. 1, 5).—In the third instar larva the appendages are absent. But the body becomes clearly segmented and the tracheal system makes its appearance. The mandibles become short, but well sclerotised. The larva has thirteen segments. The tracheal system consists of two lateral longitudinal trunks united anteriorily, but not posteriorly. There are 8 pairs of spiracles and these are present on segments 3 to 10. The spiracles are connected to the lateral longitudinal trunks by means of long

spiracular stalks. On the 11th segment only the spiracular stalk is present. It is interesting to note that the spiracular stalks of segments 3, 4 and 5 and the tracheæ that supply the head region take their origin from the lateral longitudinal trunk in segment 6. Only in segments 5 to 12 are the dorsal and ventral branches present.

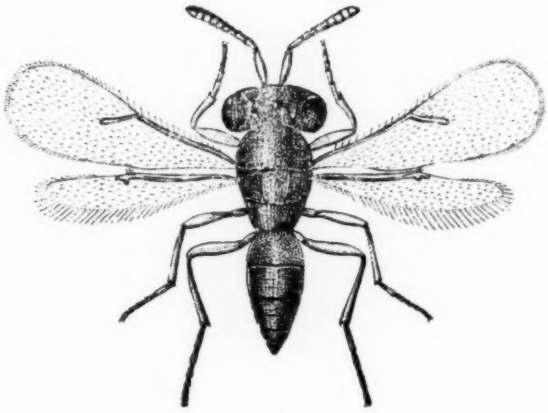

FIG. 2. *Aholcus euproctiscidis* Mani. Adult female.

The larva, now developing rapidly, devours all the egg contents. The alimentary sac becomes more prominent. It is a closed one. Now the larva secretes a thin cocoon-like protective structure around its body and prepares to pupate. The labial and the ileac glands perhaps secrete a thin viscid fluid which hardens to form the protective covering. Just before pupating the larva ejects the excrement. The duration of this stage is about 2 days.

The maximum length of the third instar was 0·66 mm. and width 0·48 mm.

Pupal Stage.—The pupa is black and imparts a black colour to the host egg. The adult parasite cuts a round hole on the egg shell and emerges.

Length of Life-Cycle.—The length of the life-cycle varied from 12 days in September (Maximum temperature 88° F., Minimum temperature 77·5° F. and Relative Humidity 75%) to 15 to 16 days in November (Maximum temperature 78° F., Minimum temperature 71° F. and Relative Humidity 60%).

Stage	*No. of days*
Incubation period of egg	1–2
First instar larva	2–3
Second instar larva	1
Third instar larva	2
Pupal stage	6–8

DISCUSSION

There is very little published record on the biology and morphology of the immature stages of this economically important group of parasitic insects. The present investigations have given some indication of their oviposition habits, larval development and the arrangement of tracheæ in the larvæ.

Oviposition.—Some members of the family Scelionidæ, including *Aholcus euproctiscidis* show preference for freshly laid host eggs for oviposition. Van Vuuren (1935) observes that in *Phanurus beneficiens* (Zehntner), an egg parasite of *Schœnobius bipunctifer* Walker, oviposition takes place only soon after egg deposition by the host female. Parman (1928) states that *P. emersoni* Girault does not oviposit in *Tabanus hyalinipennis* Hine eggs which are more than six hours old. The " teleaform " larva, which is very tiny and delicate, in all probability will not be able to pierce with its soft mandibles the body-wall of the embryo, once the body-wall becomes hardened and hence the adults prefer freshly laid host eggs for oviposition. But according to Costa Lima (1928) *Telenomus fariai* Costa Lima develops successfully in eggs of *Triatoma* whose embryos are well advanced at the time of oviposition by the parasite. Pemberton (1933) mentions that *Scelio pembertoni* Timberlake parasitises *Oxya chinensis* Thunberg eggs in all stages of development. Thus it is observed that while some members of the family Scelionidæ show a marked preference for freshly laid host eggs, others do not show such preference.

Superparasitism.—A considerable degree of selectivity is exhibited by many species of Scelionidæ. It has been observed by Morrill (1907) that the females of *Telenomus ashmeadi* Morrill are able to discriminate the parasitised host eggs from the healthy eggs by the scrapings made on the former by the ovipositing females. The same observation was made by Costa Lima in the case of *T. fariai.* Usually one egg is laid in a host egg by *Aholcus euproctiscidis.* However, in the case of *Eumicrosoma benefica* Gahan, McCulloch and Yuasa (1915) state that the females lay in eggs of *Blissus leucopterus* Say containing various stages of their own species. Noble (1935)

says that even though more than one egg of *Scelio fulgidus* Crawford in *Calataria terminifera* Walker was observed, only one reached maturity. This phenomenon was noticed in the case of *Aholcus euproctiscidis* also.

Immature Stages.—The first instar larva of *Aholcus euproctiscidis* differs from those of other species of Scelionidæ in having no antennal processes above the base of the mandibles and in having two caudo-ventral horns. The antennal processes were observed, among others, by Pemberton (1933) in *Scelio pembertoni* and Noble (1935) in *Scelio fulgidus*. In all Scelionids whose life-history has been worked out, only one caudo-ventral horn is mentioned. In some species one or two supplementary lobes are also observed. But in the case of *A. euproctiscidis* two equally developed caudo-ventral horns are present.

Only in very few cases has the second instar larva been described. This stage in *A. euproctiscidis* has no tracheal system or spiracles, but the segmentation is not discernible, as observed by Jones (1937) in the case of *Telenomus ulyetti* Nixon. McCulloch and Yuasa (1915) state that the second instar larva of *Eumicrosoma benefica* has faint segmentation of the body and possesses 4 or 5 pairs of spiracles. But the present authors are inclined to believe that this stage described by McCulloch and Yuasa is really the third instar and not the second. Many other workers have apparently observed only two instars, *e.g.*, Bakkendorf (1933) in *Phanurus angustatus* Thomson and Noble (1935) in *Scelio fulgidus*. An interesting feature of this stage is the reduction in size as compared to the first stage, which is perhaps peculiar to this group of insects alone. However, the larva attains the maximum growth in the third and the last instar.

The third instar larva is distinguished by the presence of a well developed tracheal system, the spiracles and the well defined body segments. The mandibles become short, but well sclerotised. *A. euproctiscidis* has 8 pairs of spiracles. Nine pairs of spiracles have been reported in *Telenomus ulyetti* Nixon by Jones (1937). Kamal (1938) states that in *Microphanurus basalis* Wollaston though a full complement of 9 pairs of spiracles are present, only the two pairs of thoracic spiracles are functional and the rest are minute and closed. In *A. euproctiscidis* all the 8 pairs of spiracles are well developed and functional. There are only stalks and no spiracles in the second segment. It is observed that the larva before pupating secretes a thin membranous protective covering. In the case of Encyrtids the function of ileac and labial glands in secreting a thin protective covering before the larva prepares to pupate is well known. However, the protective cover-

ing in the case of Scelionids has not been mentioned by any workers so far. Bakkendorf (1933), while studying the biology of *Phanurus angustatus*, has observed this covering and he has shown it in his figures. But he has neither described it nor named it.

SUMMARY

1. *Aholcus euproctiscidis* Mani is an important egg parasite of *Euproctis lunata* Walker, which is a serious pest of castor.

2. Freshly laid host eggs are preferred by the parasites for oviposition.

3. Usually only one egg is laid in a host egg by the parasite. In any case only one individual completes development and emerges successfully.

4. The first instar larva differs from those of other Scelionidæ, whose life-cycle has been worked out, in not possessing antennal processes and in having two caudo-ventral horns. Only one caudo-ventral horn was observed in others.

5. A definite second instar larva is present.

6. The third instar larva has a well segmented body, a well developed tracheal system and 8 pairs of functional spiracles.

ACKNOWLEDGMENT

We are grateful to Dr. B. P. Pal, Director, Indian Agricultural Research Institute, New Delhi, for providing us with the necessary facilities.

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* Not seen in original.

STUDIES IN THE ORDER PIPERALES

VI. A Contribution to the Study of Floral Anatomy of *Pothomorphe umbellata* (L.) Miq.[1,2]

BY Y. S. MURTY

(*School of Plant Morphology, Meerut*)

Received August 2, 1958

(Communicated by Prof. V. Puri, F.A.SC.)

INTRODUCTION

MEMBERS included until recently in the genus *Heckeria* Kunth. and earlier in *Piper* are now assigned by Trelease and Yuncker (1950) to a separate genus *Pothomorphe*. The embryology of the genus has attracted considerable attention especially after the much debated publication of Johnson (1902) dealing with the species *Heckeria umbellata*. Maheshwari and Gangulee (1942) after a reinvestigation of this species and Swamy (1945) from an investigation of *H. subpeltata* showed the embryo-sac to be developing after the *Fritillaria*-type and not *Adoxa*-type as was thought by Johnson. Some important embryological differences between *Piper* and *Heckeria* have also been enumerated by Johnson (1902).

Vascular anatomy of the flower of this genus has remained almost untouched all these years. Johnson (1902) made just a casual reference that there are no definite vascular bundles above the base of the fruit and that only scattered tracheids occur, especially in the region just below the stigma. Recently Nozeran (1955) showed that the *umbellate* inflorescence, which is a distinctive feature of this genus, is biparous or uniparous. The present communication deals only with the floral anatomy of one species and is based on paraffin embedded material which was made available to the author by Prof. P. Maheshwari.

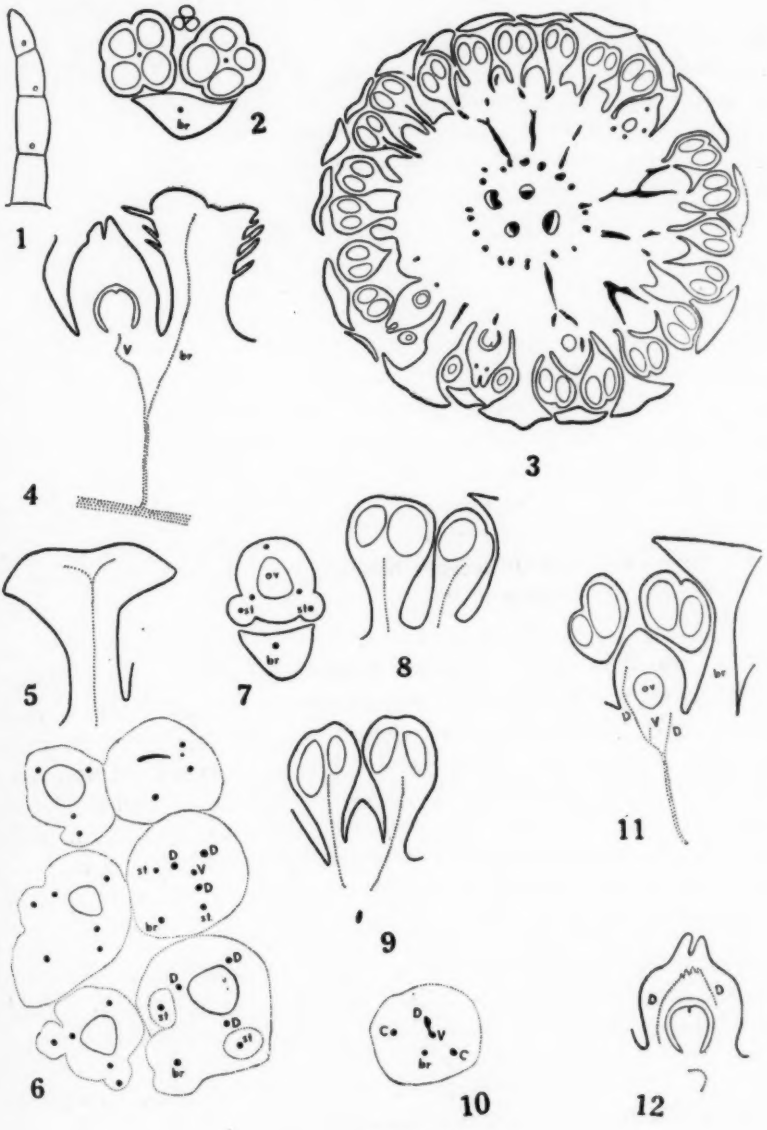
OBSERVATIONS

Pothomorphe umbellata (L.) Miq. (Syn. *Heckeria umbellata* Kunth. *Piper umbellatum* L.) is a shrub or a large herb often with large peltate leaves. The spikes are many, slender, described as *umbellate* at the end of the axillary

[1] Based on a portion of a Thesis accepted for a Ph.D. Degree of the Agra University.

[2] Research Contribution No. 19, from the School of Plant Morphology, Meerut College, Meerut.

peduncle. The sessile flowers are subtended by peltate bracts covered by long multicellular hairs (Fig. 1). Each flower has two stamens with



FIGS. 1–12

tetralocular anthers (Fig. 2), a tricarpellary gynæceum ending in three sessile stigmas and containing a single bitegmic ovule.

Floral Anatomy.—The axis of the spike has two rings of bundles, an outer with 15–20 and an inner with 4–6 (Fig. 3). A single bundle departs from the inflorescence axis supply. This diverges out horizontally to the periphery of the cortex where it gives out one branch to the bract (Fig. 4). The bract trace directly enters the organ and undergoes some branching in the shield of the peltate bract (Fig. 5). The remaining bundle reaches the base of the flower where it splits up into three branches. Two of these are antero-lateral and the third one posterior. The former splits up tangentially into two each (Figs. 6, 7). The outer of these daughter bundles are the staminal traces. Each staminal trace runs undivided within the filament of the stamen (Figs. 8, 9) and reaches the connective. On the other hand, the inner two bundles traverse the ovary wall on the antero-lateral sides. The posterior bundle, however, sends off a branch towards the chalaza (Figs. 10, 11) which is completely utilised in supplying the single ovule. The remaining outer bundle then runs in the ovary wall on the posterior side. Thus a transverse section of the ovary wall shows three bundles traversing equidistantly from one another (Figs. 6, 7). They fuse together in the upper part of the ovary just below the stigmas and finally disappear (Fig. 12). A transverse section of the flower passing through the upper regions of the ovary clearly shows the three stigmas and tetralocular anthers (Fig. 2). There is no trace of any perianth.

DISCUSSION AND CONCLUSIONS

It will be recalled that the floral vascular supply trifurcates at the base of the flower giving rise to three bundles. Of these the two anterior-lateral ones give off a branch each for the two stamens and the third posterior one sends off a branch inward that is completely utilised in supplying the single ovule. This last bundle has to be regarded as the ventral strand which is used up in furnishing a single ovular trace. The three bundles now traversing the ovary wall fuse below the stigmas and are, therefore, considered as the carpellary dorsals.

The presence of three dorsals and three stigmas in *Pothomorphe umbellata* indicate that there are three carpels in the gynæceum. The position of the carpels which can be found out from the position of the three dorsals—two anterior-lateral and one posterior—is similar to that in *Piper*. The single ovule appears to belong to one of the carpels and is regarded as lateral on account of the course of the ventral strand.

It is thus clear that *Pothomorphe umbellata* resembles very closely, the allied genus *Piper* in floral anatomy, number and position of carpels and stamens.

Summary

The floral anatomy of *Pothomorphe umbellata* is described. The bract trace and the floral trace arise conjoint. At the base of the flower the floral trace branches into three compound bundles which bifurcate into a total of six bundles. Two of these are obviously the stamen traces, another is the ventral (or placental) strand which supplies the ovular trace. The remaining three bundles have been interpreted as dorsal bundles of the carpels and hence the gynæceum has been interpreted as tricarpellary. As the ovule receives its supply from one side and not from the base it is considered to be lateral rather than basal.

Acknowledgements

The author wishes to record his grateful thanks to Prof. V. Puri for his valuable guidance and to Prof. P. Maheshwari for kindly sparing the material.

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Explanation of Figures

Figs. 1-12. Fig. 1. A single trichome. Fig. 2. Cross-section of a flower showing bract, two tetralocular anthers and three stigmas. Fig. 3. Transverse section of inflorescence showing vascular supply to flowers and bracts. Fig. 4. Flower in L.S. showing the course of vascular supply. Fig. 5. Shows the branching of the bract bundle within its shield. Fig. 6. Tangential section of inflorescence, showing flowers cut transversely and vascular supplies to the flowers. Fig. 7. Transverse section of a flower showing the separation of the stamen traces from the carpellary dorsals. Figs. 8 and 9. Show vascular supply to the stamens. Fig. 10. A part of the tangential section of the inflorescence showing separation of ventral strand from the carpellary dorsal. Fig. 11. A flower in longitudinal section showing the ventral strand and carpellary dorsals. Fig. 12. Ovary in longitudinal section showing the formation of a ring of vascular tissue below the stigmas.

Fig. 3, × 30, rest of the figures, × 60.

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A NOTE ON THE RESPIRATORY SYSTEM OF THE MALE OF *DORYLUS LABIATUS* SHUCK.

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Received July 10, 1958

(Communicated by Prof. B. R. Seshachar, F.A.SC.)

Introduction

Very little is known of the respiratory system of the Formicoidea (Hymenoptera), an earlier account by Janet (1902) of *Myrmica rubra* L. deals only with the respiratory system of gaster. The following table, based on a study of the spiracles of seventy-five species of Hymenoptera, representing twenty-five families in the classification of Imms (1957), indicates the distribution of spiracles in the various groups of adult Hymenoptera. A full account of this work is now awaiting publication.

Table I

Distribution of Spiracles in the Adult Hymenoptera

	No. of Spiracles										Type
	1	2	3	4	5	6	7	8	9	10	
Symphyta: ..	0	0	0	0	0	0	0	0	0	0	I
Apocrita:											
Ichneumonoidea	0	0	0	0	0	0	0	0	0	0	I
Evanioidea ..	0	0	0							0	II
Cynipoidea ..	0		0							0	III
Chalcidoidea ..	0		0							0	III
Proctotrupoidea	0	0	0								IV
Bethyloidea ..	0	0	0	0	0	0	0				V
Formicoidea ..	0	0*	0	0	0	0	0	0	0	0	I
Pompiloidea ..	0	0	0	0	0	0	0	0	0	0	I
Vespoidea ..	0	0	0	0	0	0	0	0	0	0	I
Sphecoidea ..	0	0	0	0	0	0	0	0	0	0	I
Apoidea ..	0	0	0	0	0	0	0	0	0	0	I

* Exception *Myrmica sabuleti* Meinert.

As will be seen from Table I some variation appears to exist within the group Formicoidea, and it was, therefore, considered desirable to study other species of this group. The genus *Dorylus* is tropicopolitan in distribution and the winged male is characterised by its large peculiar mandibles and cylindrical long abdomen. In this sex there are two thoracic, one epinotal and seven gastral pairs of spiracles. One pair of gastral spiracles lies on the pedicel and the remaining ones on the sides of the gastral tergites (Fig. 1).

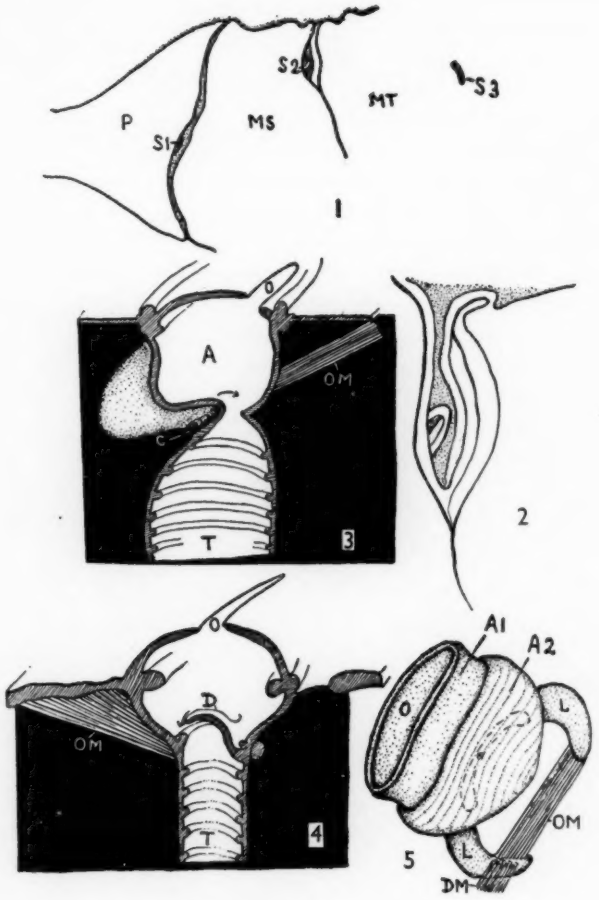
STRUCTURE AND MECHANISMS OF SPIRACLES

Anterior thoracic spiracle (Fig. 3).—Each is situated slightly ventral in position on the posterior margin of the pronotum whose internal arch encloses the spiracle. The peritreme is distinct, uniformly thickened and measures approximately ·24 mm. in length and ·11 mm. in breadth. The front lip of the spiracle is raised and slants towards the opening which forms a narrow crescentic slit. The opening leads into an atrium with thin membranous walls. The dorsal side is relatively more flexible. It is here that a sclerotised calliper is situated between the atrium and the tracheal opening. An acicular occlusor muscle, measuring approximately ·7 mm. long and 0·4 mm. broad, is attached to the calliper. The muscle arises from an apodeme of the anterior wall of the mesepisternum. The contraction of the occlusor pulls down the flexible dorsal wall to close the tracheal orifice. The opening is regained due to the elasticity of the various structures.

Posterior thoracic spiracle (Figs. 2 and 4).—Each is situated in a groove on the postero-dorsal corner of the mesepimeron. The spiracular opening has a well defined peritreme and is partially visible externally. The anterior and posterior walls project towards each other leaving a small spiracular opening in the centre. The peritreme measures approximately ·3 mm. in length and ·16 mm. in breadth. An internal peritremal lid arises from the posterior margin of the peritreme. A fan-shaped occlusor muscle is attached to this lid and takes its origin on an internal arched sclerotised ridge of the postero-dorsal corner of the mesepimeron. The muscle is approximately ·18 mm. long and ·3 mm. broad. The contraction of the muscle pulls the peritremal lid to close the spiracle. The opening is regained by the elasticity of the parts.

Propodeal spiracle.—The external opening measures approximately ·8 mm. long and ·1 mm. broad, and there is a shallow atrium which is produced into two levers. Between these lies a closing valve. The parallel-sided occlusor muscle is inserted between the apices of the two levers. A dilator muscle runs ventrally and caudally from the apex of the ventral lever.

The contraction of the occlusor pulls the closing valve forwards to close the spiracle. The contraction of the dilator and antagonistic relaxation of the occlusor opens the spiracle. Each spiracle resembles in structure and mechanism the corresponding ones described and illustrated in *Apis* by Snodgrass (1956).



FIGS. 1–5

Gastral spiracles (Fig. 5).—Each has an external opening in the form of a narrow elliptical split, measuring about ·4 mm. long and ·06 mm. broad. The peritreme is strongly sclerotised rim and leads into a primary atrium

with thick walls. It has a large number of interlacing hairs forming a good filter to prevent entry of foreign particles. The primary atrium leads into a secondary one whose walls are thin and spirally thickened. The secondary atrium is produced into two levers between which is lodged a cylindrical occlusor muscle. A similarly shaped dilator muscle runs from the ventral lever to a sternal apodeme. The contraction of the occlusor closes the tracheal opening and the dilator acts as its antagonist.

SYSTEM OF TRACHEÆ AND AIR-SACS

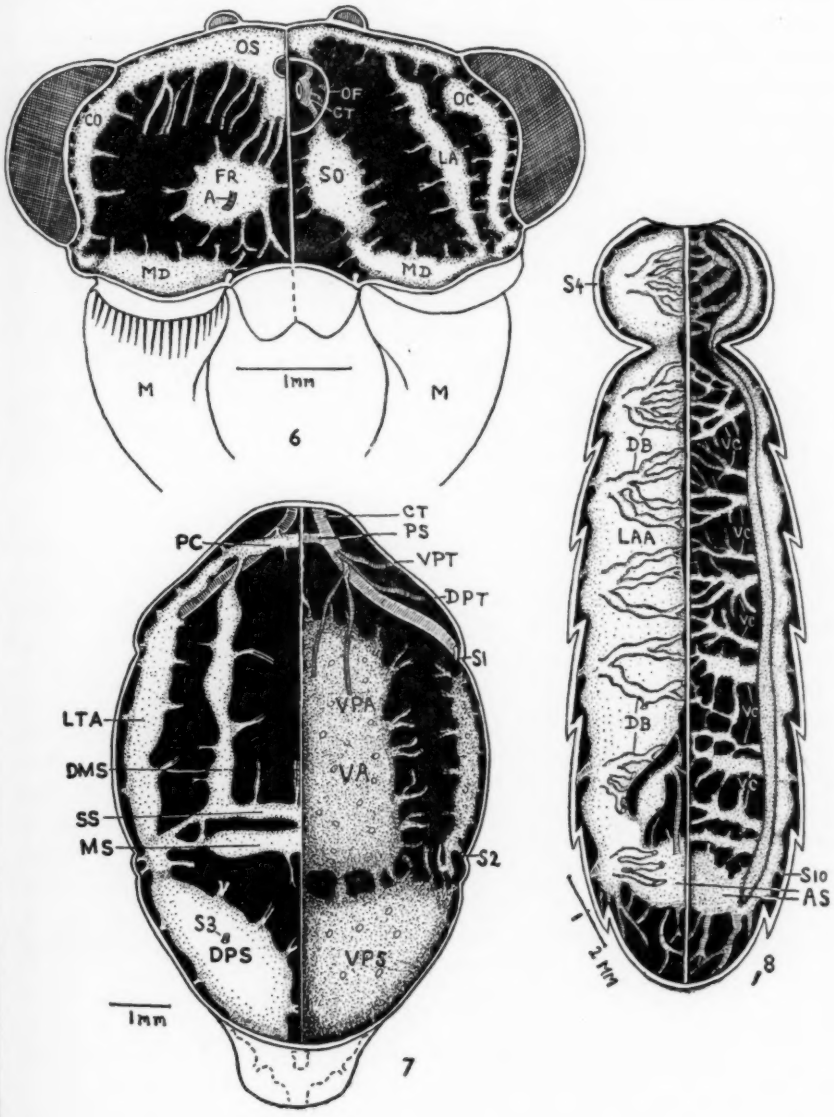
Cephalic respiratory system (Fig. 6).—A paired cervical trachea enters the occipital foramen and joins the various cephalic sacs. The dorsalmost is an ocellar sac supplying the dorsal part of the brain. An ocular branch arising from the ocellar sac divides basally into two main branches of which one forms a cerebro-optic sac supplying the frontal part of the eyes while the other ocular air-sac supplies the posterior part of the eyes. The cerebro-optic and ocular sacs give off regular transverse optic branches to the base of the eyes. The ocellar sac does not extend frontally but is connected with a small frontal sac by tubular connectives. The frontal sac gives off paired tracheæ to the antenna. The ocellar sac extends laterally after a small constriction to form the lateral sacs, which are joined to the conspicuous mandibular sacs. The subœsophageal sacs are small and appear paired.

Thoracic respiratory system (Fig. 7).—The cervical tracheæ on entering the thorax joins by a branch to the lateral thoracic sacs. The latter are fused with each other to form a large pronotal sac. The lateral air-sacs are joined dorsally and posteriorly with a single median mesoscutellar sac and a single median scutal sac both of which lie transversely. The latter gives off laterally a paired dorsal muscular sac which is joined anteriorly to the pronotal sac. The lateral thoracic sacs extend into the propodeum forming dorsal and ventral propodeal air-sacs, and the former receives short branches from the propodeal spiracles. The second thoracic spiracle receives a short trachea from the adjacent lateral sac.

The cervical tracheæ also give off branches to the various muscles, a main branch which joins the first thoracic spiracle, and a ventral posterior tracheal branch to the first pair of legs. The dorsal trachea (DPT) forms an anastomosis with the lateral sacs. The ventral posterior trachea (VPT) also joins the large median ventral thoracic sac by way of an anastomosis (VPA).

Gastral respiratory system (Fig. 8).—The constricted propodeal air-sacs form paired air-sacs on entering the petiole. After constriction, these

enter the second gastral segment and dilate to form two enormous lateral sacs. From these sacs are given out six-paired short tracheæ to the remaining



FIGS. 6–8

spiracles. The last pair of spiracular tracheæ are longer than the others. The lateral abdominal sacs also give off five or six pairs of dorsal branches in the form of an irregular saccular anastomosis. The two lateral abdominal sacs are united ventrally by means of an extremely irregular saccular anastomosis whose segmental disposition is rather difficult to make out. In the seventh segment there is a well-developed dorsal and transverse sac which forms an accessory sac near the basal ring of the genitalia. This sac extends into the genital armature and forms conspicuous sacs between the various genital organs.

DISCUSSION

In a paper, now in press, it is shown that the Hymenopteran spiracles can be classified according to the shape of their muscles. Anterior and posterior thoracic spiracles are provided with an acicular occlusor and fan-shaped occlusor muscle respectively while the abdominal spiracles are provided with parallel-shaped occlusor as well as dilator muscles. Similarly the close relationship noted between the relative development of atria and the thickness of cuticle in which the spiracles are set exists in this species as in the remaining Hymenoptera studied.

Although the plan of the cephalic respiratory system conforms with the general plan found in the other Formicoidea, there is in *Dorylus* an increase in the size of the various sacs. Such an enlargement of air-sacs and an increase in the number of connectives between them is also present in the queens of Formicoidea as compared with the workers of the same species. The thoracic respiratory system has undergone modification due to the considerable development of air-sacs which obliterate the basic pattern. The thoracic respiratory system closely resembles that of many other aculeate Hymenoptera of a similar size. In the gastral respiratory system too there is considerable variation due to the development of lateral abdominal sacs and the irregular ventral anastomosis. The occurrence of enlarged air-sacs associated with the genitalia is a feature previously noted in certain Apoidea (*e.g.*, *Megachile*, *Colletes*). The shape and size of the various parts of the body appear to have effect on the internal organisation of the tracheæ and air-sacs.

SUMMARY

The respiratory system of *Dorylus labiatus* Shuck. (♂) is described and illustrated. The classification of spiracles based on the shape of the muscles is confirmed by these findings. The results are compared with those of other Hymenoptera. It is doubtless that there exists a close relationship between

the development of number and/or size of air-sacs and the size of the body or its parts. So far as adult Hymenoptera are concerned it appears that external appearance influences the development of internal tracheal system.

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Explanation of Figures

Figs. 1–5. Fig. 1. Lateral view of the thoracic sclerites in relation to the spiracles. Fig. 2. Position of the posterior thoracic spiracles as seen from outside. Fig. 3. Schematic sectional view of the anterior thoracic spiracle to show the closing mechanism. Fig. 4. Diagrammatic sectional view of the posterior thoracic spiracle to show the closing mechanism. Fig. 5. Internal side view of the third gastral spiracle.

P = Pronotum; *MS* = Mesepimeron; *MT* = Metapleuron; $S_1 - S_3$ = Spiracles; *A* = Atrium; *T* = Trachea; *OM* = Occlusor muscle; A_1–A_3 = Subdivisions of atrium; *D* = Lid; *O* = Spiracular opening; *L* = Lever; *DM* = Dilator Muscle; *C* = Calliper.

Figs. 6–8. Fig. 6. Frontal (left) and posterior (right) view of the cephalic respiratory system. Fig. 7. Dorsal view of the thoracic respiratory system. Dorsal air-sacs of the right side are removed to expose the ventral air-sacs. Fig. 8. General system of tracheæ and air-sacs of the abdomen as seen from above. Right lateral air-sac is cut to expose the irregular ventral commissures.

OF = Occipital foramen; *CT* = Cervical trachea; *OS* = Ocellar air-sac; *CO* = Cerebro-optic air-sac; *FR* = Frontal air-sac; *A* = Antennal trachea; *MD* = Mandibular air-sac; *LA* = Lateral air-sac; *SO* = Subœsophageal air-sac; *OC* = Ocular air-sac; *M* = Mandible. *PC* = Pronotal air-sac; *LTA* = Lateral thoracic air-sac; *DMS* = Dorsal muscular air-sac; *SS* = Scutal air-sac; *MS* = Mesoscutellar air-sac; *DPS* = Dorsal propodeal air-sac; *VPS* = Ventral propodeal air-sac; *VA* = Ventral thoracic air-sac; S_1–S_3 = Spiracle and spiracular trachea; *CT* = Cervical trachea; *PS* = Pronotal commissure; *VPT* = Ventral posterior trachea; *VPA* = Ventral posterior anastomosis; *DPT* = Dorsal posterior trachea. S_4–S_{10} = Spiracle and spiracular trachea; *LAA* = Lateral abdominal air-sac; *DB* = Dorsal branches; *VC* = Ventral Commissures; *AS* = Accessory air-sac.

SPORE-FORMS IN SPOROPHORES OF *GANODERMA LUCIDUM* (LEYSS.) KARST.

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Introduction

Perhaps it is worthwhile to place on record that although considerable work has been done on basidiospores of *Ganoderma lucidum* (Leyss.) Karst., informations regarding the production of secondary spores and gasterospores are very few. Buller (1933) and Bose (1935) mention that fruit-bodies of *Ganoderma* produce secondary spores. Bose studied *Ganoderma lucidum* and *Ganoderma applanatum* and he referred to those spores as secondary spores which are produced by the tramal hyphæ and are found in sporophores collected from Nature. Buller (1933) called them secondary basidiospores since these are asymmetrically produced at the ends of hyphæ and are shot out by drop-excretion mechanism. But none of them observed the formation of secondary spores from thin-walled hyphæ in the fruit-bodies of *Ganoderma* obtained in culture.

That *Ganoderma* also produces gasterospores have been recorded by a few workers. Bose (1933), while working with *Ganoderma lucidum*, claimed to have seen the gasterospores not only in the pore-tubes but also in the context and trama. The existence of gasterospores in fruit-bodies of *Ganoderma* had also been noted by Pilat (1936). But none of them said anything about their detailed characteristics excepting in that they are round, thick-walled and verrucose. In order to throw more light on various spore-forms in *G. lucidum*, the present investigation was undertaken.

Materials and Methods

The materials were collected from Calcutta and suburbs during the wet months of July to September 1953–56. The fungus appeared in the form of bracket-shaped fructifications (sometimes stipitate) on several hosts, but only those fruit-bodies which were found to grow on living trunks of *Swietenia mahagoni*, were collected. The fructifications usually appeared on the trunks about one to two feet above the soil. The specimens were carefully selected

and for further study, they were brought to the laboratory, examined and studied while in a fresh condition. For the study of secondary spores and gasterospores, fruit-bodies were produced in artificial culture (Banerjee and Sarkar, 1956), from secondary mycelium obtained by mating compatible primary mycelia of opposite sex. In making microscopic preparations, all sections were cut by free-hand sectioning method (Banerjee, 1935), mostly from fresh materials. For temporary mounting and staining lactophenol-cotton-blue gave good results. Sections were also mounted in either warm lactophenol or lactic acid and observed without staining.

Observations

Basidiospores

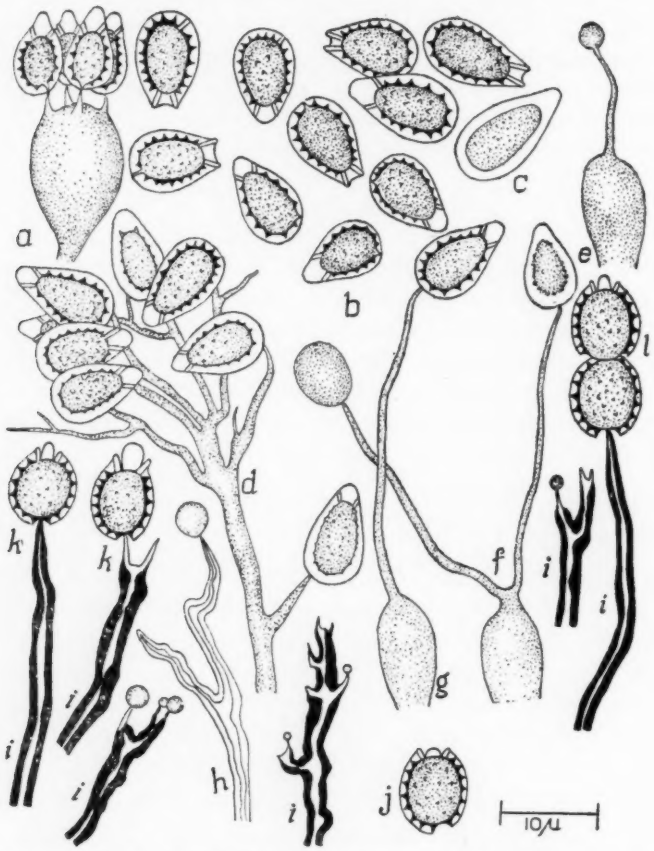
The basidiospores are ellipsoid or ovoid and each with a hyaline epispore projecting at the apex to form a papilla-like structure which collapses at maturity giving the spore a truncate appearance. The endospore is yellowish-brown, apparently smooth when young but becoming faintly to strongly punctate or somewhat roughened at maturity. The dimension of the basidiospores is about 10–12 × 6–8 μ (Text-Fig. 1, *a–b*).

Besides normal basidiospores, two other types of spores have also been found within the pore-tubes of the fruit-bodies of *G. lucidum*. The characteristic features of each type have been described as follows:

(*a*) *Secondary spores.*—At maturity, these resemble the basidiospores in morphological characters so closely that in a group of spores it becomes very difficult to distinguish one from the other. Like the basidiospores, mature secondary spores are also ovoid with truncated apex, brown in colour and with hyaline epispore, but the endospore is apparently smooth or slightly punctate. The dimension of the spores also agrees closely with that of the basidiospores and it is 9–11×6–8 μ (Text-Fig. 1, *c–g*).

These secondary spores have been found in the sporophores, obtained either from Nature or from culture. A section through the pore-tubes of a sporophore produced in culture never shows the hymenium. Instead, the pore-tubes are lined internally with numerous thin-walled, hyaline hyphæ, having dense protoplasmic contents and possessing clamp-connexions. The hyphæ are either simple or profusely branched at the apices. Secondary spores, which are usually terminal, are cut off directly by these thin-walled hyphæ or their branches. Formation of several spores by the branches of a single hypha in a close and aggregated manner often gives a clustered appearance (Text-Fig. 1, *d*).

Sporophores collected from Nature in dry weather during the rainy season often produce secondary spores but in a different way. A section through the pore-tubes of such a sporophore shows but very few mature



TEXT-FIG. 1. Different spore-forms of *G. lucidum*: *a–b*, Basidiospores; *c–g*, Secondary spores; *h–l*, Gasterospores.

basidia. Instead, they are lined internally by immature basidia and numerous long tramal hyphæ are found to project into the cavities of the pores. Very often, the apices of these immature basidia become narrowed and elongated to form thin-walled living hyphæ (Text-Fig. 1, *e–g*). They are similar in appearance with the tramal hyphæ. The original tramal hyphæ and also those formed by the transformation of the basidia are found to produce secondary spores terminally in the same way as they are produced

by the thin-walled hyaline hyphæ in the sporophores produced in artificial culture.

(*b*) *Gasterospores.*—These spores, more accurately called 'Gasteroconidia', have been observed in the sporophores which were collected from Nature during a long period of dry weather in the growing season. They have also been found in the hymenial regions of the sporophores of *Ganoderma lucidum*, produced in culture. They are always borne by the highly thick-walled, hyaline or dark-brown hyphæ within the pore-tubes, often partly filling the cavities. Terminally or from the sides of these hyphæ short, hyaline narrow, pointed, peg-like structures develop on which these spores are borne (Text-Fig. 1, *h–i*). Like basidiospores, gasteroconidia are also brown in colour and double-walled with verrucose endospore. But they are generally spherical or more or less subglobose in shape and provided with two usually opposite notches in the exospore. The apical notch is guarded on the two sides by two prominent projections of the endospore so that a more or less funnel-shaped depression is formed. Within the depression of the apical notch is formed a small papilla-like structure, which sometimes becomes considerably enlarged (Text-Fig. 1, *j–k*). The gasteroconidia are generally borne singly, but sometimes two conidia may be found situated one above the other (Text-Fig. 1, *l*). The width of a gasterospore varies from 6–8 μ.

Summary

1. In this paper information regarding the various spore-forms of *Ganoderma lucidum* has been presented.

2. It has been found that besides the normal basidiospores, two other spore types also occur in the fructifications of the fungus collected from Nature or produced in artificial culture. These are 'secondary spores' and 'gasterospores'.

3. Characteristic features of all the spore types have been fully described and their modes of origin noted.

4. All the three types of spores are more or less identical to each other in appearance. But the secondary spores have endospores less verrucose than the endospores of the other two types, whereas the gasterospores are characterised by two opposite notches in their exospores.

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SUGARCANE MITES—A REVIEW

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Introduction

Mites (Acarina) are minor pests of sugarcane. Hirst (1912) recorded a new species of mite (*Tarsonemus bancrofti*) on sugarcane in Barbados. Another mite, *Tarsonemus spinipes* Hirst, has been reported by him (1913) as fairly common in several of the West Indian Islands. Jones (1914) identified the mite feeding on sugarcane stalks at Puerto Rico as *T. spinipes* and another found on sugarcane leaves as *Tetranychus* sp. Walcott (1921) recorded *Damacus mitens* from Puerto Rico. Hirst (1923) recorded *Paratetranychus indicus* sp. nov., at Coimbatore on Sorghum. Later (1926), he also described a new species, *Schizotetranychus andropogoni* on Sorghum. Cherian (1931) also recorded *Paratetranychus indicus* on cane in South India. Holloway (1936) noted *Tetranychus exsiccator* Zhnt. in Hawaii, Philippines and Java and *Tarsonemus spinipes* in Hawaii, Peru and West Indies. Ingram (1937) made a record of *T. bancrofti* Mich., on sugarcane for the first time in U.S.A. Rahman and Sapra (1940) and Khan and Bhatia (1940) recorded *Paratetranychus indicus* Hirst as a pest of sugarcane in various parts of India. The symptoms of damage noted by Rahman and Sapra, however, differed from those described by Khan and Bhatia. According to Parthasarathy (1950) the red mite (*P. indicus*) did not occur in South India, but he noted another white species of mite (*Paratetranychus* sp.). Rao (1951, 1952) recorded *Schizotetranychus andropogoni* Hirst in South India. Khanna and Butani (1954 *a*, 1954 *b*) opined that there might be more than two species of sugarcane mites among those recorded so far in India. Box (1953) has listed among the species of mites *Paratetranychus indicus* from India; *Tarsonemus spinipes* from Barbodos; *Paratetranychus* (*Oligonychus*) *viridis* Banks and *Tarsonemus bancrofti* from Cuba; *Tarsonemus spinipes* and *Tetranychus exsiccator* from Hawaii (immigrant): *Eriophyes* (*Phytoptus*) sp., *Tarsonemus bancrofti* and *Tetranychus exsiccator* from Java (the last one also from Jamaica); *Pediculopsis* sp. from Natal; *Tarsonemus spinipes* from Peru; *Tetranychus exsiccator* from Philippines; *Paratetranychus* (*Tetranychus*) *sacchari* Meg., *P.* (*Oligonychus*) *viridis* and *Tarsonemus spinipes* from Puerto Rico;

Tarsonemus bancrofti and *Tatranychus telarius* L. from Queensland, *Glyciphagus domesticus* Deg., *Paratetranychus simplex* Banks, *P.* (*Oligonychus*) *viridis*, *Tarsonemus bancrofti* and *Uropodus* sp. from U.S.A. and *Tyroglyphus sacchari* Banks from West Indies. Muthukrishnan (1956) recorded an eriophyid mite (unidentified) in South India.

MORPHOLOGY

Hirst (*loc. cit.*) gave a very brief description of the species recorded by him. Cherian (1933) briefly described the various stages of *Paratetranychus indicus*. Rahman and Sapra (1940 *a* and *c*) have studied in detail the morphology of all the stages of *P. indicus*. MacGregor (1942) described the adults of both sexes of *P. sacchari*. Rao (1952) reproduced the description of *S. andropogoni* given by Hirst.

BIOLOGY

Cherian (1933, 1938) studied the bionomics of *P. indicus* and observed its life-cycle to occupy 9–12 days. Egg, larval, nymphal and pre-oviposition periods were 1–3, 3–4, 2–4 and 1–2 days respectively. Rahman and Sapra (1940 *a*, *b*) also studied the biology of *P. indicus*. According to them, pre-oviposition and oviposition periods were 1–2 days and 14·5–19·5 days respectively, whereas post-oviposition and longevity periods ranged between 1–3 days in summer and 2–6 days in winter, 8–12 days in summer and 12–20 days in winter respectively.

NATURE OF DAMAGE

Mites have been recorded a feeding on all parts of the sugarcane plant-leaf, stalk and roots. *Uropodus* sp. found only in U.S.A. has been recorded attacking roots of sugarcane.

Graywood (1919) observed that *Tarsonemus spinipes* attacked principally the stalk and leaf-sheaths where it formed large clusters of very small, flat brown blisters that gave the plant tissue a scabby or scarred appearance. The mite infested new plants by migrating upward from the infested seed pieces. Colonies of this mite lived in cane tops underneath the green leaf-sheaths. He further observed that due to attack of *P. viridis*, minute whitish sand grains appeared on the leaf which were the empty cart skins of the immature stages. The red spots on the leaves were apparently not due directly to the feeding of the mites but to the fact that the lesions caused by their feeding allowed the entrance of the spores of red rot *Colletotrichum* which caused the reddening of the leaves. Pemberton (1929) studied in detail the possibility of *T. spinipes* being a carrier of node galls. The results

of his experiments, though unsatisfactory, favoured the belief that these mites were not the causative agents of node galls. Martin (1938), however, observed that *T. spinipes* was usually present on stalks bearing by galls. Healthy canes failed to produce galls when plant juices extracted from galls were injected into them or when placed in contact with galled varieties, or when directly inoculated with diseased tissues. Isolation studies failed to indicate any causal organism. On certain varieties that normally produced galls, no galls appeared when repeated applications of sulphur were made to the cane tops. Ingram (1937) observed *Tarsonemus bancrofti* between the leaf-sheath and the stem.

In India attack by mites is limited to leaves only. According to Rahman and Sapra (1940) the leaves affected by *Paratetranychus indicus* turned red, gradually dry up and fall off. Khan and Bhatia (1946) observed that the leaves attacked by *P. indicus* became dry and straw coloured instead of red. Khanna and Butani (1954) recorded symptoms of both these types separately in South and North Bihar respectively.

Incidence

Wolcott (1921) observed in Puerto Rico that after an extended drought, *P. viridis* was very abundant on the lower leaves of young canes, but after the drought was broken by several heavy rains the mite disappeared. Khanna and Bandyopadhyay (1950) in an attempt to fix up an adequate sample size for estimating the incidence of mites observed that the incidence varied in intensity even in a plot of 1/40th acre and that sampling requirements were different for patches of varying degrees of intensity. Scrutiny of the variations revealed that, in cases of low incidence (less than 0·6 puparia per square inch), nested sampling was not necessary. Sampling might be done on the basis of leaves alone, 15 leaves from each plot of 1/40th acre being sufficient to bring down the error of estimation well within 5% of the mean. For infestations between 0·5 and 1·55, 40 random leaves from 10 units of three feet each from 1/40th acre plot was sufficient. Puttarudriah (1956) and Agarwal (1957) observed an increase in population of *S. andropogoni* with a rise in temperature.

Alternate Host Plants

According to Rahman and Sapra (1940 *a*), *Paratetranychus indicus* is widely distributed all over India. In South India, it has been reported on sugarcane, *Andropogon sorghum, Panicum javanicum* and *P. distachyum.* In Punjab besides sugarcane, *P. indicus* also feeds on *Sorghum vulgare* and *S. halepense.* Khan and Bhatia (1946) added three more host plants for

this mite from Sind (Pakistan), *viz.*, *Digera arvensis*, *Sesbania ægyptiaca* and *S. grandiflora*. Cherian (1951) and Murthy and Krishnamurthy Rao (1956) recorded the mite, *S. andropogoni* on *Dicanthium annulatum* in South India.

VARIETAL RESISTANCE

Rahman and Sapra (1940) observed that sugarcane varieties with soft leaves were more susceptible to the attack of *P. indicus* as compared to those with hard and tough leaves. Khan and Bhatia (1946) recorded incidence in 15 improved varieties, including Co. 213, Co. 312, Co. 313 and Co. 450 and observed that there was little or no difference in the intensity of attack. Khanna and Ramanathan (1947) studied the relation between the nature of leaf surface and attack by mites. They pointed out that the presence of stomatal grooves between the veins provided shelter for the mites and the spinous outgrowth afforded a suitable held for spinning the webs. Khanna and Butani (1954 *a* and *b*) studied the powers of resistance of sugarcane varieties to mite attack and concluded that under the pressure of severe infestation there was no difference in the behaviour of sugarcane varieties to mite attack.

CONTROL

A good deal of work has been done all over the world on the control of mites including those attacking sugarcane. As regards mechanical control measures, Cherian (1933) suggested that edges of fields should be kept clear of wild food plants, such as the grasses (*Panicum javanicum* and *P. distachyum*). Ingram *et al.* (1939) suggested that canes to be transported from infested areas should be soaked in water at 115° F. for half-an-hour as a measure against mites (*T. bancrofti*).

The chemical control of mites is a problematic one, because of the fact that the span of their life is very short and there is always a sufficient population in the egg stage to survive the effect of a particular chemical used which is usually not an ovicide. On the other hand, the various parasites and predators of these mites are completely wiped off by the miticide used. These parasites and predators do not have such a reservoir of eggs, to refill their depleted ranks, with the result that when the mites hatch out from the surviving eggs, they find the environment most favourable for flourishing as the agents of biological control in nature are no more there. This necessitates the use of a miticide which should either have exceptionally long residual effect, or it should be an ovicide as well. Such a miticide is yet to be found. The work so far done on this aspect has shown that use of sulphur or Folidol

against these mites is quite effective. In the *Kew Bulletin* (1890) it is suggested that canes affected by disease caused by mites should be well cleaned and immersed for 24 hours in water and carbolic acid. Then these should be soaked for a few minutes in milk of lime and spread in the sun to dry for one day before planting. Rutherford (1913) struck a note of caution while pointing out that sulphur was effective only when the infested surfaces of the plant were exposed to direct sunshine for some time during the day or to intense reflected heat. Dry sulphur should be applied when the leaves are wet with dew or, failing this, they should receive a preliminary spraying with water. He also suggested spraying sulphur with hydrated lime as the latter caused the sulphur to adhere to the leaves. Cherian (1933) suggested dusting with flowers of sulphur and road dust (1 : 4). He further added that the dusting was useless when the infestation had spread and damage has been caused. Later (1938), he mentioned that application of sulphur was generally effective against mites. He also recommended a spray of 1 lb. fish-oil rosin soap in 6 gallons of water per acre. It is also reported elsewhere (1939) that lime sulphur with at least 18% sulphur content gave 100% mortality of mites but had no effect on eggs. If some sodium salt of a sulphated organic compound like Aresket was added as a spreader, it formed a thin film round the eggs and then the eggs do not hatch. One part of chemical in 2,600 parts of lime sulphur brought about 90–100% mortality of eggs. Ayyar (1940) also recommended application of flowers of sulphur or spraying with lime sulphur solution. Metcalf (1948) opined that chlorinated hydrocarbons are relatively ineffective in controlling the phytophagous mites. Later (1951) he reported that, of the insecticides in common use today, only the dinitrophenols and organic phosphorous toxicants are of practical value as Acaricides. Agarwala and Prasad (1951) conducted laboratory trials and pointed out that Rhodiatox, Toxaphene and BHC were best for the control of mites, DDT also proving quite good. But Brown (1951) mentioned that DDT caused an increase of population of *Paratetranychus* and other mites. Jepson (1952) had however reported certain exceptions here DDT had proved effective against citrus bud mite (*Aceria sheldoni*). According to Asquith and Kane (1952) and Leink *et al.* (1952) DDT or 2, 2-*bis* (*p*-chlorophenyl)-1, -1 dichloroethane possess acaricidal properties. Agarwal (1957) tried Folidol (0·02%), Malathion (0·1%), Ekatin (0·1%), Pestox-3 (0·035%), Endrin (0·035%) and DDT+BHC (0·24%) and noticed that barring Folidol, the toxicity of all other insecticides petered out by 6th day. Further, he recorded an increase in population of mites in plots treated with Endrin, Malathion and DDT + BHC. Prasad and

Prasad (1957) also reported the superiority of Folidol, in controlling mites.

As regards biological control, the various natural enemies recorded by Cherian (1933) include, *Scymnus gracilis* Mostsch., *Scolothrips sexmaculatus* Perg., *Liophæna gracilipes* Sharp (*Oligota flaviceps* Sharp), *Orius* (*Triphleps*) *tantilus* Motsch. and *Geocoris* sp. Rahman (1940) recorded *Scymnus gracilis* Most., as a predator of *Paratetranychus indicus*. This predator remains active during March to November and hibernates as an adult during December to February. During its active period the life-cycle is completed in 7–49 days with as many as 16 broods. The beetle prefers to feed on eggs as resting stages of the mite. In the laboratory at 33° C. adults of *S. gracilis* were found to feed, on an average, on 99 eggs and the larvæ on 60–200 eggs (depending upon the age of larvæ) daily. Khan and Bhatia (1946) also recorded the larvæ and adults of this predaceous beetle on all stages of *P. indicus*, the predator being active all the year round. Box (1953) mentioned *Podothrips lucasseni* Krug. on *Tarsonemus spinipes* in Hawaii and *Stethorus vagans* Blackb. on *T. spinipes* and *Tatranychus exsiccator* in Java and Hawaii.

FUTURE LINES OF WORK

A well planned survey of the various States should be taken up with a view to finding out the exact number of species of mites that feed on sugarcane.

A detailed systematic study of *Tetranychus*, *Paratetranychus* and *Schizotetranychus* and allied genera and identification of the Indian forms are the problems that need be taken up immediately. At present even the exact number of species occurring in India is not known. Morphology and biology have been studied by couple of workers, but then, the life-history and bionomics always vary under different atmospheric conditions from State to State and from tract to tract. These fundamental studies need be investigated in detail.

The nature of damage done by this pest has been studied, but the extent of damage has still to be known. The ecological studies in relation to the extent of damage need also to be undertaken. Attempts have been made to study the varietal resistance of sugarcane to the attack of mite, but this aspect too should be pursued further to confirm the relationship between the morpho-anatomical structure of sugarcane leaves and the attack of the pest.

As regards control also, little work has been done except a few laboratory trials. Field experiments with such of the miticides that have shown promis-

ing results under laboratory conditions need be taken up. Besides, a search for the natural enemies, with a view to establishing the same, will be of immense use, in eradicating this pest.

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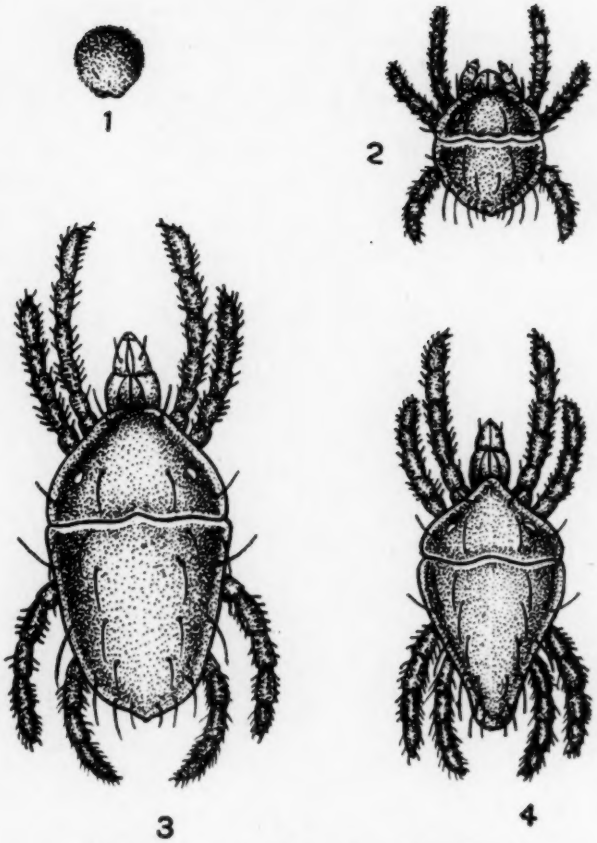

Sugarcane Mite (*Paratetranychus indicus* Hirst.)
1. Egg. 2. Nymph. 3 & 4. Adults (♀ & ♂).

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AMINO ACIDS DURING THE DEVELOPMENT OF *ORYZIAS MELASTIGMA* (McCLELLAND)

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Needham (1955) has pointed that the most important feature of organ and tissue differentiation at the biochemical level is the synthesis of specific proteins and that for understanding this problem a prerequisite is a thorough knowledge of the movement of the amino acids, whether in free, peptide or protein form in the eggs and embryos during normal development.

The first attempt to study the amino acids during development was by Holtfreter, Koszalka and Miller (1950) who found that there is a surprising similarity in the total amino acid composition of all the egg types, developmental stages and germ layers investigated by them and that in the amphibian egg neither do new amino acids appear nor do the others existing already disappear. It was therefore concluded by these authors that embryonic chemodifferentiation does not occur at the level of amino acids. Eakin, Berg and Kutsky (1950) were also unable to find any marked change in free amino acids during the early development of *Hyla regitta* and *Rana pipiens.* Later Kutsky, Eakin, Berg and Kavanau (1953) also found that there was no over-all pattern of change. Chen and Rickenbacker (1954) also found more or less similar results in a study of the free amino acids in the development of *Triton palmatus* and *Triton alpestris.*

Subsequent investigations by other authors showed that the conclusions of Holtfreter *et al.* (1950) could not be final and were much too pessimistic as Needham stated (1955).

The results of other investigators differ from the above conclusions. Berg (1950) found that in the development of *Strongylocentrotus purpuratus*, Alanine, Glutamine and Lysine progressively declined. Gustafson and Hjelte (1950, 1951) studied the amino acid content in the development of *Paracentrotus lividus* and found that there was little change during cleavage but during gastrulation there was a drop in the free and a concomitant rise in the peptide amino acids. Kavanau (1953) found that in the development of sea urchin the free amino acids fall into several groups with regard to quantitative changes but have no consistent pattern of change with regard

to the protein-bound amino acids, which decline continuously until hatching when a sharp drop occurs. Later Kavanau (1954) found in his studies of *Paracentrotus lividus* cyclic changes in protein-bound amino acids, indicating alternative periods of yolk degeneration and tissue protein synthesis.

Deuchar (1956) studied the free amino acid in the developing tissues of the embryo of *Xenopus lævis*. In a later paper (1958) she studied the free amino acids during the cleavage stages of *Xenopus lævis* and found an increase in the concentration of Glutamic acid and a decrease in free Aspartic acid.

In view of the contradictory nature of the findings of several investigators, it appears worthwhile to make a thorough study of the amino acids during the development of animals of different groups, before we can formulate a generalisation. Neither the free nor protein-bound amino acids have been investigated so far in the development of any fish. The present study relates to the protein-bound amino acids in the development of the fish *Oryzias melastigma* (McClelland). The chief events of morphogenesis and organogeny of the different stages are also mentioned. The causal relationship between morphogenetic events and changes in amino acid distribution is not discussed in the present account, as this work is in progress.

Material and Methods

The protein-bound amino acids were studied by circular and two-dimensional chromatographic techniques using Whatman Filter-Paper No. 1. For the circular paper chromatography filter-paper with 13 cm. diameter was used, and for two-dimensional chromatography 19×19 cm. paper was used. The procedure adopted by Giri *et al.* (1952) has been followed for the preparation of total acid hydrolysates, and for each stage of development 50 eggs were subjected to hydrolysis. The solvent system consisted of *n*-butanol, acetic acid and water mixed in the ratio of 4: 1: 1, v/v, and 0·1% ninhydrin in 95% acetone w/v was used as colour developing reagent. The process of multiple running was resorted to and the desired separation of the overlapping amino acids was obtained. The individual amino acids were identified by running mixed chromatograms of known and unknown amino acids under identical experimental conditions and with reference to the R_f values of the known amino acids.

Results

Seventeen amino acids were recognised in all, in the chromatograms, *viz.*, Leucine and Isoleucine, Phenylalanine, Valine, Methionine, Proline, Tyrosine, Alanine, Threonine, Glycine, Serine, Glutamic acid, Aspartic

acid, Histidine, Arginine, Asparagine, Ornithine and Cystine. Of these seventeen amino acids the following show variations during the embryonic development: Methionine, Tyrosine, Histidine and Ornithine.

1. During cleavage and gastrulation stages sixteen amino acids are present, Ornithine alone being absent.

2. At about 24 hours of development, while the axiation and tubulation become established in the embryo, Methionine and Histidine are lacking, the remaining fifteen amino acids being present.

3. At 48 and 54 hours of development only fourteen amino acids are present, Methionine, Histidine and Ornithine being absent. During the 48-hour-stage of development the embryo gets well defined primary constrictions in the anterior part of the nerve tube, the optic vesicles are well developed and the ectoderm thickens to form the rudiment of the lens. The 'heart' also begins to pulsate. During the 54 hours of development the midgut begins to coil and the lateral line sense-organs arise as ectodermal invaginations.

4. At 72 hours of development, the embryo develops the rudiments of the pectoral fins and also branchial pouches. The amino acid composition of this stage is the same as in the 24 hours of development.

5. During the 96 hours of development Histidine and Ornithine disappear, the other fifteen amino acids being present. In this stage a slight reduction in the size of the yolk is noticed and the liver arises as a ventral diverticulum of the gut.

6. At 120 hours of development Ornithine reappears while Methionine disappears. The amino acid composition of this stage is similar to that of 24 and 72 hours of development of the embryo. During this stage the embryo twists and turns frequently inside the egg and is heavily pigmented. The air-bladder arises as an outgrowth of the pharynx.

7. The 144 hours stage has the same amino acids as the 48 and 54 hours of development.

8. Methionine and Ornithine reappear at 168 hours of development while Tyrosine and Histidine disappear. During this stage the mouth opening is established and the first gill-cleft establishes an opening to the exterior and thymus rudiment arises as a thickening of the branchial arches.

9. Methionine, Tyrosine, Histidine and Ornithine are absent during the last stage of the developing embryo, namely, 192 hours. The embryo has grown fully and exhibits vigorous movements. Five branchial arches

TABLE I

Amino acids during the development of Oryzias melastigma

Stages	Amino acids																			Patterns
	Leucine	Phenylalanine	Valine	Methionine	Tryptophane	Proline	Tyrosine	Alanine	Threonine	Glycine	Serine	Glutamic acid	Aspartic acid	Histidine	Arginine	Asparagine	Lysine	Ornithine	Cystine	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1. Cleavage ..	A	B	C	D	..	F	G	H	I	J	K	L	M	N	O	P	..	..	S	I
2. Gastrula ..	A	B	C	D	..	F	G	H	I	J	K	L	M	N	O	P	..	..	S	II
3. 24 hours ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	..	R	S	III
4. 48 ,, ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	.	..	S	III
5. 54 ,, ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	..	..	S	II
6. 74 ,, ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	..	R	S	IV
7. 96 ,, ..	A	B	C	D	..	F	G	H	I	J	K	L	M	..	O	P	..	..	S	II
8. 120 ,, ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	..	R	S	III
9. 144 ,, ..	A	B	C	..	..	F	G	H	I	J	K	L	M	..	O	P	..	..	S	V
10. 168 ,, ..	A	B	C	D	..	F	..	H	I	J	K	L	M	..	O	P	..	R	S	VI
11. 192 ,, ..	A	B	C	..	..	F	..	H	I	J	K	L	M	..	O	P	..	..	S	

For convenience of ready reference the amino acids have been indicated by the letters of the alphabet.

are well developed supplied with blood vessels and an appreciable reduction in the size of the yolk is noticed.

TABLE II

Distribution of different patterns in different stages of development

Pattern	Present in stages	Characterised by the absence of
I	1 and 2	Ornithine
II	3, 6 and 8	Methionine and Histidine
III	4, 5 and 9	Methionine, Histidine and Ornithine
IV	7	Histidine and Ornithine
V	10	Tyrosine and Histidine
VI	11	Methionine, Tyrosine, Histidine and Ornithine

The protein-bound amino acids during the development of *Oryzias* show six different patterns of amino acid distribution, each of which is distinguished from the other by the presence or absence of a few amino acids.

Pattern I is present in the cleavage and gastrulation stages of the developing embryo. Sixteen amino acids are present in this pattern, Ornithine alone being absent.

Pattern II is characterised by the absence of Methionine and Histidine, the other 15 amino acids being present. The embryo in its 24, 72 and 120 hours of development has this type of pattern and it is interesting to observe the alternation in occurrence of this pattern in successive stages of development.

Pattern III is characterised by the absence of Methionine, Histidine and Ornithine and this pattern is present during the 48, 54 and 144 hours of the developing embryo.

Pattern IV occurs during the 96 hours of development. Fifteen amino acids are present in this pattern, Histidine and Ornithine being absent.

Pattern V is present in 168 hours of development. Tyrosine and Histidine are absent in this pattern.

In pattern VI Methionine, Tyrosine, Histidine and Ornithine are absent and this pattern occurs in the 192 hours of the developing embryo.

SUMMARY

1. The distribution of protein-bound amino acids, during the development of *Oryzias melastigma*, has been made qualitatively by paper chromatographic technique.

2. The protein-bound amino acids show six different patterns of distribution during the development of the embryo.

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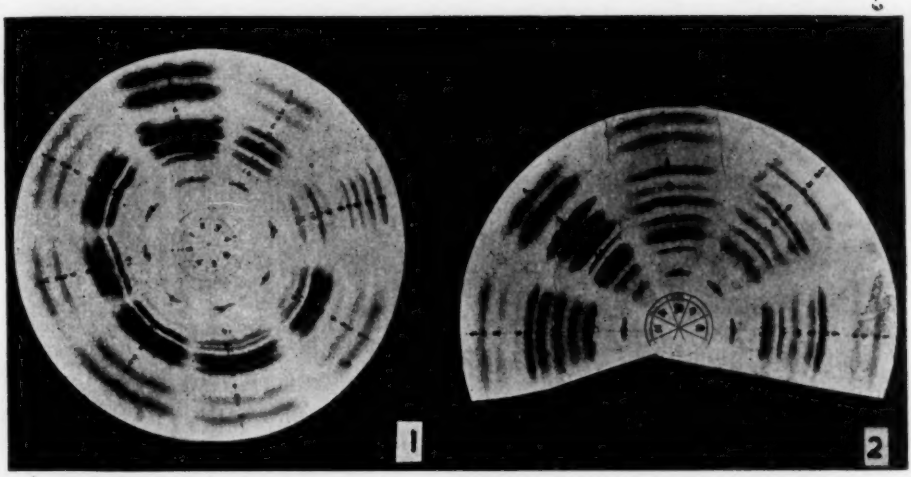

FIG. 1. Chromatogram of the Protein-bound amino acids during the development of *Oryzias melastigma*. The following stages are represented: 1, 3, 4, 5, 6, 7 and 8.

FIG. 2. Chromatogram of the protein-bound amino acids during the development of *Oryzias melastigma*. The following stages are represented: 2, 9, 10 and 11. *K. Known amino acids.*—Nos. 1 to 11 in the inner circle indicate the different stages of development. *Amino acids.*—1. Leucine and Isoleucine, 2. Phenylalanine, 3. Valine and Methionine, 4. Tryptophane, 5. Tyrosine, 6. Proline, 7. Alanine, 8. Threonine, 10. Glycine, Serine and Aspartic acid, 11. Asparagine, 12. Arginine, 13. Histidine, 14. Lysine and Ornithine, 15. Cystine.

ON THE CULTURAL METHODS OF DIFFERENTIATION OF *RHIZOBIUM* FROM *AGROBACTERIUM RADIOBACTER*

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Agrobacterium radiobacter is the most frequent concomitant organism found with rhizobial isolations made from nodules. Further, it is difficult to distinguish it from the rhizobia because of its similarity in general appearance as well as certain cultural characters (Palacios and Bari, 1936). Certain cultural tests are still of some use in distinguishing it from *Rhizobium*. Litmus milk, plain milk, potato slants, yeast extract, mineral salts medium with $CaCO_3$ *plus* (*a*) 1% dextrin, (*b*) 1% inulin, yeast extract mannitol broth pH 10, and pH 11–12, Koser's uric acid medium, etc., are some of the media used for the purpose.

During the course of our studies on root nodule bacteria in Gujarat soils, the above media were utilized to differentiate the rhizobial isolates from *A. radiobacter*. Allen and Allen (1950) have considered these to be reliable. We found however that our isolates did not behave in a particular fashion and the results obtained by us which are at variance with those reported below inasmuch as undoubtedly raise a question whether certain methods reported are reliable for distinguishing the isolates obtainable under our conditions.

Isolation of the Bacteria from the Nodules

Thirty-one isolates of nodule bacteria were isolated from the healthy nodules of different species of several host plants by the usual methods on yeast extract mannitol congored (1 : 20,000) and crystal violet (1 : 80,000) agar, and were tested in the following media for their cultural differentiation from *A. radiobacter*. The purity of culture was tested by all criteria.

1. *Growth of the isolates in plain milk, litmus milk and on potato slants*

Methods for differentiation between *A. radiobacter* and the nodule bacteria were first suggested by Löhnis and Hansen (1921). They found that potato slants and litmus milk were useful for the purpose. *A. radiobacter* produced a brown colouration of the medium, while *Rhizobium* did

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not. In the opinion of other workers, however, the coloration produced by certain strains of *Rhizobium* and lack of colouration on the part of certain strains of *A. radiobacter* were sufficient to confuse results in many cases, with the result that soil bacteriologists are reluctant to use these media. Hofer (1941) years later reported that *A. radiobacter* grew very similarly to *Rhizobium* except that it produced a browning of the medium; either slightly or distinctly. In some cases the browning was so little that the cultures could not be distinguished. When the cultures of rhizobia were compared 10 to 20% were of indefinite and uncertain reaction.

Our observation also show that these media give variable reactions and cannot be relied upon (Table I).

2. *Growth characters of the isolates in yeast extract mannitol broth at pH* 10 *and pH* 11–12

Hofer (1935), after testing a number of media for differentiation of rhizobia from radiobacter, reported comparative usefulness of (1) moderately alkaline and (2) strongly alkaline liquid media for the purpose. According to him, when inoculated into moderately alkaline medium, *A. radiobacter*, produced growth without exception. All rhizobia except *R. meliloti* could grow in this medium. In the case of strongly alkaline medium too all the cultures of *A. radiobacter* could grow while all rhizobia except two cultures of *R. meliloti* could not grow in this medium.

On being tested for their ability to grow in these media, our isolates were variable in their ability to grow. The individual reaction of each isolate is tabulated in Table I. It can be seen therefrom that isolates Nos. 2, 3, 11, 14, 15, 16, 17, 18_1, 18_2, 19, 21, 28 and 31 grow in the moderately alkaline medium. The isolates Nos. 14, 17, 19, 21, 28 grow in strongly alkaline medium as well. All these isolates belong to different cross-inoculation groups. This indicates the presence of *A. radiobacter* in these cultures. Other cultural characters of these isolates are also very similar to those of *A. radiobacter* and hence it can be definitely stated that these isolates have been contaminated by *A. radiobacter*. However, all those isolates growing in the moderately alkaline medium cannot be said to have been contaminated as their other cultural characters do not agree with those of *A. radiobacter*.

Rhizobium meliloti isolates from lucerne (Nos. 24 and 24_2) however failed to grow in both these media, contrary to the findings of Hofer. It might be that these strains cannot tolerate alkalinity.

3. *Change in reaction of yeast extract mineral salts medium with* $CaCO_3$ plus (*a*) 1% *dextrin*, (*b*) 1% *inulin*.

Fred *et al.* (1932) reported the production of acid reaction in yeast extract mineral salts medium *plus* 1% dextrin by *A. radiobacter*, while rhizobia give alkaline reaction in the same medium. Later on Georgi and Ettinger (1941) confirmed the same. Inulin was also found to give similar reaction by the same workers.

The reactions exhibited by our isolates in these media are tabulated in Table I. It can be seen that the isolates giving positive reaction for rhizobia elsewhere did not produce any change in reaction in the case of dextrin, while some even produced acid. Certain isolates gave acid reaction in both the carbohydrates. Isolates Nos. 15 and 16 gave acid reaction in both the media. Isolate No. 12 produced no change in dextrin while acid in inulin. This indicates the presence of *A. radiobacter* in these cultures; but their hebaviour in other media for differentiation from radiobacter is however characteristic of rhizobia. These cultures were again found to be producing nodules on the host plants under aseptic conditions (1955). As such these media cannot be relied upon for the purpose of differentiation of *Rhizobium* from *A. radiobacter*.

4. *Growth characters of the isolates in Koser's uric acid medium*

Hofer (1941) reported that after eight days' incubation at 25° C. in Koser's uric acid medium, cultures of *A. radiobacter* produced a pellicle or heavy ring, while cultures of *Rhizobium* failed to grow or did so without producing a pellicle affording thus a valuable test for differentiation. Our isolates, however, were found to be variable in their ability to grow in this medium. Isolates Nos. 13, 15, 17, 18_1, 18_2, 19, 21 could grow without pellicle, while isolates Nos. 2, 3, 12, 16, 22, 22 *a*, 22_1, 22_2, produced slight turbidity. Only one isolate No. 28 from ' lang ' produced characteristic pellicle of *A. radiobacter*. This isolate gave all the reactions of radiobacter.

From Table I it is not possible to judge definitely that a particular isolate (except No. 28) is contaminated with *A. radiobacter* as the reactions are variable and confusing. For further study isolates Nos. 1, 4, 25 and 29 (from pea), No. 20 (from *Tephrosia purpurea*), No. 22 (from *Zornia diphylla*), 24 (lucerne), 27 (berseem), which gave all the cultural tests positive for true rhizobia, were selected for isolates Nos. 12, 15 and 16 obtained from ' shewri ' bean and ' guar ' respectively did not give uniformly positive tests either for rhizobia or radiobacter. All the isolates belong to different bacterial plant cross-inoculation groups and were therefore put to further tests by following cultural methods used for differentiation from radiobacter.

TABLE I

Growth characters of the isolates on different media for differentiation from A. radiobacter

Sl. No.	Isolate No.	Host plant	Source	Reaction in plain milk	Reaction in litmus milk	Growth on potato slants	Yeast ext. mineral salts with $CaCO_3$ *plus*		Yeast ext. mannitol broth		Koser's uric acid medium
							1% dextrin	1% inulin	pH 10	pH 11–12	
1	1	Pea (*Pisum sutivum* L.)	Poona Agr. College	S.Z.	b. S.Z.	–	b	b	–	–	–
2	2	do.	do.	No S.Z.	do.	+	0	b	++	–	++
3	3	do.	Field-Institute	Browning	a, S.Z.	++	0	0	++	–	+
4	4	do.	Poona Agr. College	S.Z.	b, S.Z.	–	0	b	–	–	–
5	5	do.	Field-Institute	Sli. brown	a.	+	b	b	–	–	–
6	6	do.	do.	S.Z.	b, S.Z.	–	b	b	–	–	–
7	7	do.	Poona Agr. College	S.Z.	do.	–	0	b	–	–	–
8	8	do.	do.	S.Z.	do.	+	b	b	–	–	–
9	9	do.	do.	S.Z.	do.	+	b	b	–	–	–
10	10	do.	Field Institute	S.Z.	do.	++	a	a	–	–	–
11	11	do.	do.	Sli. brown	do.	++	0	0	++	–	–
12	12	'Shewri' (*Sesbania ægyptiaca*)	Institute area	Sli. brown	do.	++	0	a	++	–	+
13	13	'Kulthi' (*Dolichos biflorus* L.)	do.	clot	a, clot	+	b	b	–	–	–
14	14	Dioncha (*Sesbania aculeata*, Poir.)	do.	clot	do.	+ brown	a	a	++	+	++
15	15	Bean (*Dolichos lablab* L.)	do.	Sli. brown	b, S.Z.	+	a	a	++	–	++

16	16	'Guwar' (*Cyamopsis tetragonoloba* Taub.)	(Institute area	S.Z.	b, S. Z,	+	a	a	++	–	+
17	17	'Chawli' (*Vigna catjang*, Walp.)	do.	clot	a, clot	++ brown	a	a	++	–	++
18	18_1	Sunnhemp (*Crotalaria juncea* L.)	do.	clot	do.	++ brown	0	b	++	–	++
19	18_2	do.	do.	clot	do.	++ brown	0	b	++	–	++
20	19	Groundnut (*Arachis hypogæa* L.)	do.	Brown	b, S.Z.	++ brown	0	b	++	+	++
21	20	*Tephrosia purpurea*	do.	S.Z.	do.	+	b	b	–	–	–
22	21	*Desmodium rotundifolium*	do.	clot	a, clot	++ brown	a	0	++	+	++
23	22	*Zornia diphylla*	do.	S.Z.	b, S.Z.	+	b	b	–	–	+
24	22 *a*	do.	do.	S.Z.	do.	+	b	b	–	–	+
25	22_1	do.	do.	S.Z.	do.	+	b	b	–	–	+
26	22_2	do.	do.	S.Z.	do.	+	b	b	–	–	+
27	23_2	'Tur' (*Cajanus cajan*)	do.	S.Z.	do.	+	a	a	–	–	–
28	24_1	Lucerne (*Medicago sativa* L.)	do.	S.Z.	do.	+	b	b	–	–	–
29	24_2	do.	do.	S.Z.	do.	+	b	b	–	–	–
30	25	Pea	do. Previous year's inoculated plot	S.Z.	do.	–	b	b	–	–	–
31	26	do.	do.	S.Z.	do.	–	b	b	–	–	–
32	27	Berseem (*Trifolium alexandrinum*)	do.	No S.Z.	b	–	b	b	–	–	–
33	28	'Lang' (*Lathyrus sativus* L.)	Broach soil	Browning	a, brown	++ brown	a	a	++	++	++ P
34	29	Pea	do.	S.Z.	b, S.Z.	–	b	b	–	–	–
35	30	'Lang'	Institute area	S.Z.	do.	+	b	b	–	–	–
36	31	*Crotalaria medicagone*	do.	S.Z.	do.	+	0	0	+	–	–

0 = no change in reaction
a = acid
b = alkaline

S.Z. = Serum zone
P = Pellicle
Sli. = slight

– = no growth
+ = slight growth
++ = good growth

5. *Growth on mannitol calcium glycerophosphate agar*

Plates of this medium (Stap and Ruschmann, 1924) have been used (Riker *et al.*, 1930) for distinguishing *A. radiobacter* from *Phytomonas tumefaciens* and *Phytomonas rhizogenes*. On this medium colonies of *A. radiobacter* are raised and slimy and surrounded by a brown halo with an outer zone of white precipitate consisting probably of tricalcium phosphate. Slants of the above medium have been suggested for use in distinguishing *A. radiobacter* and *Rhizobium* spp. from each other, inasmuch as the former is said to produce a distinct browning of the medium, while *Rhizobium* spp. do not (Sarles *et al.*, 1935).

None of the eleven isolates mentioned before produced any browning of the medium. The outer zone of white precipitate was also absent indicating thereby that our isolates were true rhizobia. Isolate No. 28 however produced distinct browning of the medium with white precipitate around the growth.

6. *Growth on crystal violet glycerol nitrate soil extract agar*

Leonard as quoted by Fred *et al.* (1932) has reported that *A. radiobacter* produces large colonies with deep blue or violet centres and clear rims on this medium. Colonies of rhizobia are small and evenly coloured throughout.

On the plates of this medium all the eleven isolates produced small evenly coloured colonies typical of rhizobia. The isolate No. 28 which gave all the reactions of radiobacter formed colonies with violet centres and clear rims, thereby confirming its identity as *A. radiobacter*.

7. *Production of H_2S (ZoBell's Method)*

Hofer tested various cultures of rhizobia and *A. radiobacter* by ZoBell's method (1943) in liquid medium containing tryptone and 1% mannitol and found that vigorous production of H_2S by radiobacter, whereas the cultures of *Rhizobium* did not grow after two days or would grow slightly without forming a pellicle and/or H_2S. Freshly isolated cultures of true rhizobia will not produce H_2S except on long incubation while fresh cultures of radiobacter show vigorous H_2S production.

All the eleven isolates gave negative test for H_2S by ZoBell's method while the isolate No. 28 which was confirmed as a radiobacter gave strongly positive reaction for H_2S.

The most reliable and final test for differentiating the rhizobial isolates from *A. radiobacter* is the test for their ability to produce nodules on the host plants under aseptic conditions. All the eleven isolates imentioned

were able to produce nodules on the host plants under aseptic conditions (1924).

Discussion

It can be seen from Table I that the isolates exhibited wide variation in cultural reactions for differentiation of rhizobia from *A. radiobacter*. The same isolate gives certain tests positive for radiobacter, while in other tests it gives reactions characteristic of rhizobia. Growth characters of the isolates in the two alkaline media (1. pH 10 and 2. pH 11–12), in yeast extract mineral salts medium *plus* (*a*) dextrin, (*b*) inulin, and Koser's uric acid medium have been advocated by the workers as reliable tests for differentiation between *Rhizobium* and *A. radiobacter*. Our isolates were not found to behave uniformly in these media. Only one isolate, No. 28, from ' lang ' gave all the positive tests for radiobacter. All the isolates growing in moderately alkaline medium cannot be considered to have been contaminated with radiobacter, as their cultural reactions in some other media are characteristic of nodule bacteria. However, the isolates growing in the strongly alkaline medium may be considered contaminated with radiobacter as rhizobia are not known to tolerate so much alkalinity except *R. meliloti*. Hofer (1935) could distinguish between rhizobia and radiobacter by using these two media except *R. meliloti* which could grow in both the media. Table I is self-explanatory in this respect. Isolates Nos. 14, 17, 19 and 21 (none of which is *R. meliloti*) were growing in both the media. This indicates the presence of *A. radiobacter* in their cultures. It is also noteworthy at the same time that the same isolates were found to be growing in Koser's uric acid medium without pellicle, whereas radiobacter is known to grow with heavy ring or pellicle. The reactions of these isolates in dextrin and inulin are also not uniform, making the differentiation difficult. Only one isolate (No. 28) gave all the cultural tests positive of *A. radiobacter*. Isolates Nos. 1, 4, 6, 8, 9, 20, 22, 22 *a*, 22_1, 22_2, 24, 25, 27, 29 and 30 gave all the cultural tests positive of rhizobia.

Wide variation has been noticed in the reactions of the isolates in yeast extract mineral salts medium with $CaCO_3$ *plus* (*a*) dextrin and (*b*) inulin. In these media rhizobia give alkaline reaction while radiobacter gives acidic. It can be noted from Table I, that isolates Nos. 18_1, 18_2 and 19, give typical alkaline reaction characteristic of rhizobia, but their other cultural reactions are similar to that of radiobacter. Isolates Nos. 2, 3 and 31 which give all other positive reactions of rhizobia have failed to produce any change in reaction in the case of dextrin while isolates Nos. 15 and 16 which have given acidic reaction in both these carbohydrates while their reactions are

typical of rhizobia in strongly alkaline yeast extract mannitol broth (pH 11–12), Koser's uric acid medium, mannitol calcium glycerophosphate agar, crystal violet glycerol nitrate soil extract agar and negative test for H_2S by ZoBell's method. Isolate No. 12 also produces no change in reaction in dextrin while acid in inulin. Its other cultural characters are typical of rhizobia like Nos. 15 and 16. All these isolates were able to form nodules on the host plants under aseptic conditions. This is considered to be the most reliable and final test for confirmation of rhizobia.

From the above observations one may be led to believe that *A. radiobacter* might be growing along with *Rhizobium* in the isolates Nos. 12, 15 and 16. As their cultural characters are typical of rhizobia in most of the media except (1) moderately alkaline medium, (2) yeast extract mineral salts and (*a*) 1% dextrin, (*b*) 1% inulin; and their ability to produce nodules on the host plants leave no doubt about their identity as true rhizobia. Thus it may reasonably be concluded that these two media cannot be depended upon for the purpose of differentiation of our rhizobial isolates from radiobacter, though other workers have recommended and relied upon them. In Koser's uric acid medium most of the isolates (except No. 28) found to have been contaminated, have failed to form pellicle which is characteristic of radiobacter. However none of the true rhizobia have shown any variation. So it may be useful along with other media. The growth characters of the isolates in (1) mannitol calcium glycerophosphate agar, (2) crystal violet glycerol nitrate soil extract agar, and ability to produce H_2S by ZoBell's method, agree with the observation of original workers uniformly and hence may be confidently depended upon for our isolates.

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NOTES ON SOME FUNGI FROM SOUTH INDIA—VII

By T. S. Ramakrishnan, F.A.Sc.

Received October 6, 1958

***Pythium vexans* de Bary**

On the stem of *Hevea brasiliensis* M. Arg. causing canker, Kanjirapally (Kerala), 15-9-1955, T.S.R.

This disease was prevalent on three-year old plants in a budwood nursery in the rainy months of the south-west monsoon period. From minute cracks in the bark drops of latex had oozed out and dried as black streaks on the surface. The diseased bark was depressed and later infested with boring beetles. Below the bark the tissues had turned brown, the discoloration extending to the wood in some instances. Linear spread was either extensive or limited.

The infection was noticed in different regions of the plant. The upper portions of the tap-root of the root-stock, the collar region or the stem of the scion were affected in discontinuous or extensive patches. The latex flowing out of the affected portions had an offensive odour. The leaves often exhibited a bronzed appearance.

P. vexans was repeatedly isolated from the diseased bark. Oospores were produced in abundance in agar cultures. Sporangia were rare on solid media but were developed readily when bits of culture were floated in water. Successful infection was obtained when healthy shoots were inoculated through wounds. This fungus (as *P. complectins* Br.) has been reported to be responsible for bark canker on rubber in Malaya also (Sharples, 1937). In India this pathogen has been recorded on other hosts (Ramakrishnan, 1948) but this is the first report on rubber.

***Uncinula religiosa* sp. nov.**

Mildew patches white, epiphyllous, at first isolated and circular later coalescing and covering the whole surface; conidia formed in chains, hyaline, oblong or barrel-shaped, 30–38 × 14–20 μ. Ascocarps numerous, epiphyllous, spherical, dark brown, 75–90 μ in diameter, appendages hyaline, uncinate, 16–20 in number and 140–170 μ long; asci 4–6 in each ascocarp, hyaline, ovate, 45–60 × 28–35 μ, often evanescent; ascospores usually 4 in each ascus, oblong, hyaline, 24–28 × 10–15 μ.

Maculæ albæ, epiphyllæ, primo separatæ atque circulares, tum coalescentes atque operientes totam paginam; conidia efformata in catenulis, hyalina, oblonga vel doliiformia, 30–38 × 14–20 μ. Ascocarpi plures, epiphylli, sphærici, fusce brunnei, 75–90 μ diam., appendicibus hyalinis, uncinatisque, 16–20 numero, 140–170 μ longitud.; asci 4–6 in singulis ascocarpis, hyalini, ovati, 45–60 × 28–35 μ, sæpe evanescentes; ascosporæ ut plurimum 4 in singulis ascis, oblongæ, hyalinæ, 24–28 × 10–15 μ.

On living leaves of *Ficus religiosa* L. (Moraceæ), Glenburn Estate, Nilgiris, 12–3–1958, T.S.R.

This mildew was severe on this estate and caused heavy defoliation of even young leaves. Numerous ascocarps were seen as minute dark round bodies embedded in the white mildewy growth. But on storage these are usually shed so that in the herbarium specimens they may be missing after six months. The appendages are simple and uncinate at the apex. The wall is thickened in the lower half of the appendage and thin in the upper portion.

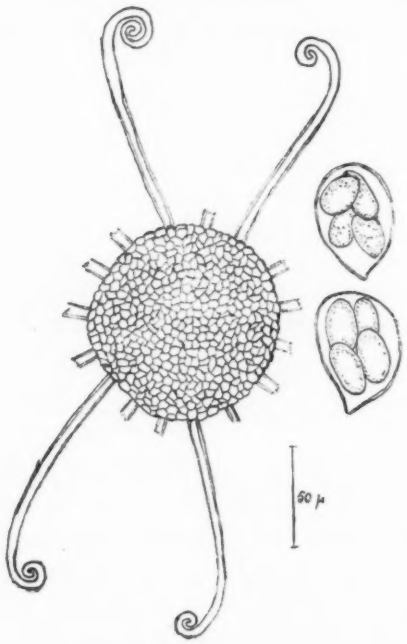

Fig. 1. *Uncinula religiosa* sp. nov.—Ascocarp and asci.

***Aecidium travancoricum* sp. nov.**

Infected portion yellow, indefinite, slightly convex on the upper surface; pycnia mainly epiphyllous, sometimes hypophyllous, minute, dome-shaped, subcuticular, reddish orange when young, 40–80 μ high and 250–300 μ wide; æcia hypophyllous, cupulate, irregularly gregarious, deep-seated, 270–300 μ wide and up to 500 μ high; peridia whitish, lacinate, reflexed, peridial cells globose to rhomboid, thick-walled, verrucose, subhyaline to brown according to age, 30–37 $\times$ 28–32 μ; æciospores catenulate, globose to subglobose, thin-walled smooth, 25–35 $\times$ 25–28 μ and yellowish brown.

Pars infecta lutea, indefinita, paulum convexa in pagina superiore; pycnia ut plurimum epiphylla, minuta, cupulæformia, subcuticularia, juvenilia quidem rubro-aurantiaca, 40–80 μ alta, 250–300 μ lata; æcia hypophylla, cupulata, irregulariter gregaria, alte infixa, 270–300 μ lata et usque ad 500 μ alta; peridia albidia, laciniata, reflexa, cellulis peridialibus globosis vel rhomboideis, crassis parietibus præditis, verrucosis, subhyalinis vel brunneis pro ætate, 30–37 $\mu \times$ 28–32 μ; æciosporæ catenulatæ, globosæ vel subglobosæ, tenuibus parietibus præditæ, leves, luteo-brunneæ, 25–35 $\mu \times$ 25–28 μ.

On living leaves of *Coffea travancorensis* W. & A. (Rubiaceæ), 28–5–1958, Kottayam, T.S.R.

The pycnial and æcial stages of the rust have been observed. The affected leaves could be easily recognised by the raised yellowish patches conspicuous among the dark green foliage. *Aecidium nobile* Syd. was originally recorded on *Coffea arabica* from Mysore (Sydow, H. & P. and Butler, 1906). Later Mundkur and Thirumalachar (1946) who re-examined the specimen have reported that the host plant was not *Coffea* but *Pavetta* and have made the rust a synonym of *A. pavettæ* Berk. The present host is definitely *C. travancorensis* and the rust is different.

***Pellicularia filamentosa* (Pat.) Rogers**

Rogers, D. P. 1943, *Farlowia*, **1**, 95–118.

On living leaves and twigs of *Lagerstræmia indica* (Lythraceæ), Mundakayam (Kerala), 25–5–1958, T.S.R.

The host is cultivated as an ornamental plant. During the south-west monsoon many of the branches are affected by the fungus. Some of the leaves are blighted by the formation of large indefinite water-soaked brownish lesions. On other leaves which remain green a white powdery growth of the fungus develops on the lower surface. On this growth the basidia and

basidiospores are formed. This is a new host for the fungus which is widely prevalent in this region.

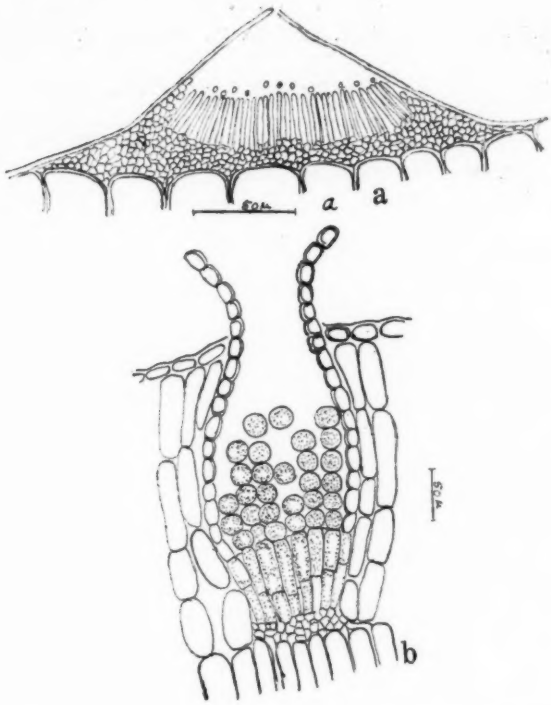

FIG. 2. *Aecidium travancoricum* sp. nov. (*a*) Section of pycnium, (*b*) Section of æcium.

***Trametes corrugata* (Pers.) Bres.**

Syn., *T. persoonii* (Fr. ex Cooke) Pat.

On stumps of *Hevea brasiliensis* M. Arg. (Euphorbiaceæ), Pathanapuram (Kerala), 15–9–1957, T.S.R.

Numerous sporophores develop on the stumps of trees cut down for replanting. Evidently it grows as a saprophyte only. No instance of actual death of rubber trees due to infection by this fungus has been observed.

I am indebted to Dr. S. N. Banerjee, Professor of Botany, Calcutta, for the identification of *Trametes*. Rev. Fr. H. Santhapau of St. Xavier's College, Bombay, has kindly made the Latin translation of the diagnoses of new species.

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ABSENCE OF SECRETION OF ACETYLCHOLINE ON STIMULATION OF NERVES IN AN UNSTRIATED MUSCLE

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The views about chemical transmission at neuromuscular junctions are well known. It is generally believed that such transmission takes place by secretion of acetylcholine, adrenaline or noradrenaline. This idea of neurohumoral transmission has appeared unsatisfactory to some and acetylcholine has been assigned other roles besides its possible action at neuromuscular junctions. According to Nachmansohn (1955), the mechanisms of conduction and transmission are essentially similar. The rhythmic activity of the heart is in some way linked with the enzymatic synthesis of acetylcholine (Burn, 1950). Jullien, Ripplinger and Cardot (1954) believe that acetylcholine is just a product of metabolism which must be removed. They believe that acetylcholine has no motor function.

During estimations of acetylcholine in the stomach muscle of the frog, *Rana tigrina*, it was noticed that though the muscle contained considerable quantities of acetylcholine, none was detected in the eserinised saline in which the muscles were immersed and oxygenated at room temperature. It was therefore decided to investigate the liberation of acetylcholine in this muscle.

Methods

These experiments were performed on the stomach muscle of the frog *Rana tigrina*. The animal was stunned with a blow on the head and rapidly pithed. The stomach was removed along with its nerves and its mucous membrane was gently removed avoiding any injury as far as possible. It was then aerated for 15 mintues in ordinary Ringer's solution and then in eserinised Ringer's solution for two hours. The muscles were removed and the acetylcholine extracted. The Ringer's solution in which the muscles were immersed was tested for acetylcholine.

For testing the effect of stimulation, the muscle along with its nerves was laid in a petri dish on a pair of electrodes and stimulated with strong induction shocks. It was then immersed in 12 c.c. of eserinised Ringer's solution in a beaker and aerated for one hour. Any fluid sticking to the petri dish was washed into the beaker. The aeration was done to allow any acetylcholine liberated to diffuse into the Ringer's solution.

To test the effect of potassium, the muscle was aerated as above and then immersed in a strong solution of potassium chloride, 0·15 M, for 10 minutes and then quickly washed to remove the excess of potassium chloride sticking to the muscle, so that it may not affect the frog's rectus or leech muscle, and then aerated for one hour in eserinised Ringer's solution. Such a strong solution of potassium chloride is a powerful stimulant of the muscle. The Ringer's solution was then tested for acetylcholine.

The effect of calcium was similarly tested, the muscle being immersed in 0·1 M calcium chloride for 15 minutes.

Acetylcholine in the muscle was estimated, using the frog's rectus by the method similar to that used by Richter and Crossland (1945) for brain tissue. After experimentation, the muscle was immediately transferred to a stoppered bottle kept in a freezing mixture, and was allowed to freeze. Eserinised Ringer's solution was prepared and brought to pH 4 by adding N/10 HCl. Twenty c.c. of this was heated to a temperature of 95–100° C. in a water-bath and the weighed muscle transferred to it. It was cut into very small pieces while it was kept at this temperature for 5 minutes. The mixture was decanted, allowed to cool and then centrifuged for 10 minutes. The deposit was stirred in a few c.c. of pH 4 eserinised Ringer's solution and centrifuged again. The supernatant fluid was collected, brought to pH 4, and kept in a refrigerator.

Before testing, the extract was brought to pH 7 to 7·4 with N/10 NaOH. The extract was further diluted with eserinised Ringer's solution, so that 1 c.c. of the extract was equivalent to 100 mg. of the tissue. The assay of acetylcholine was carried out on eserinised frog rectus and sensitive leech preparation against solutions of known acetylcholine concentrations, avoiding errors of sensitisation as suggested by Feldberg (1948).

RESULTS

The acetylcholine content of 10 muscles respectively was 5·9, 5·2, 5·1, 5·3, 4·7, 4·6, 6·7, 5·2 and 4·2 μg./g. of tissue. This is less than that found in mammalian smooth muscle. All of this was intracellular, as shown by immersion in Ringer's solution.

Effect of immersion in Ringer's solution.—In 20 experiments, muscles weighing 2·5–3 g. were immersed and aerated in eserinised Ringer's solution for 2 hours. No acetylcholine could be detected in the solution. This is in agreement with results of Chujyo (1952), who found that no acetylcholine diffused out of guinea pig's intestines under similar conditions. If the muscles were stretched, still there was no liberation of acetylcholine. The frog's stomach muscle thus differs in this respect from guinea pig's intestines, which liberate acetylcholine when stretched (Chujyo, 1952).

Effect of electrical stimulation.—In 20 experiments stimulation of the muscle and its nerves, with induction shocks, did not liberate any acetylcholine. The frog's stomach muscle becomes less excitable if its initial length decreases, and if it is aerated in saline, it gradually shortens to a constant length, and may become inexcitable to electric current. Under such conditions only nerves will be stimulated. Such differential stimulation did not result in the liberation of any acetylcholine, so that neither the stimulation of muscle nor its nerves liberate any acetylcholine.

Effect of depolarisation.—Excess of potassium is known to produce depolarisation of muscle or nerve membranes. In 16 experiments, strong solutions of potassium chloride, such as 0·15 M, failed to liberate any acetylcholine. Similarly in 16 muscles, strong solution of calcium chloride, such as 0·1 M, failed to liberate any acetylcholine. Thus the membrane of the frog's stomach muscle appears to be very resistant, or acetylcholine is very strongly bound.

Effect of heating.—If the muscle, is heated to 50° C. then acetylcholine is liberated in traces in 1 hour. If heated to 60° C., then there is marked liberation of acetylcholine. This shows that acetylcholine comes out of the muscle cells only when the membrane is damaged. The contractile mechanism also undergoes change when heated to 50–60° C., as it relaxes actively (Singh and Singh, 1954). The contractile proteins are denatured and possibly they release the bound acetylcholine.

The extract of the muscle produced by heating to 60° C., causes frog's stomach muscle to contract and then relax (Fig. 1). The contraction is presumably due to acetylcholine and relaxation to adrenaline or noradrenaline. Frog's stomach muscle is quite sensitive to adrenaline; in sensitive muscles, 1 in 20 million produces appreciable relaxation. No relaxing substance was found in the solution surrounding the muscle after immersion for 2 hours on stimulation with induction shocks.

Acetylcholine was also liberated from the muscle if treated with eserinised Ringer's which some ether had been added. Change in osmotic pressure of the saline did not produce any liberation.

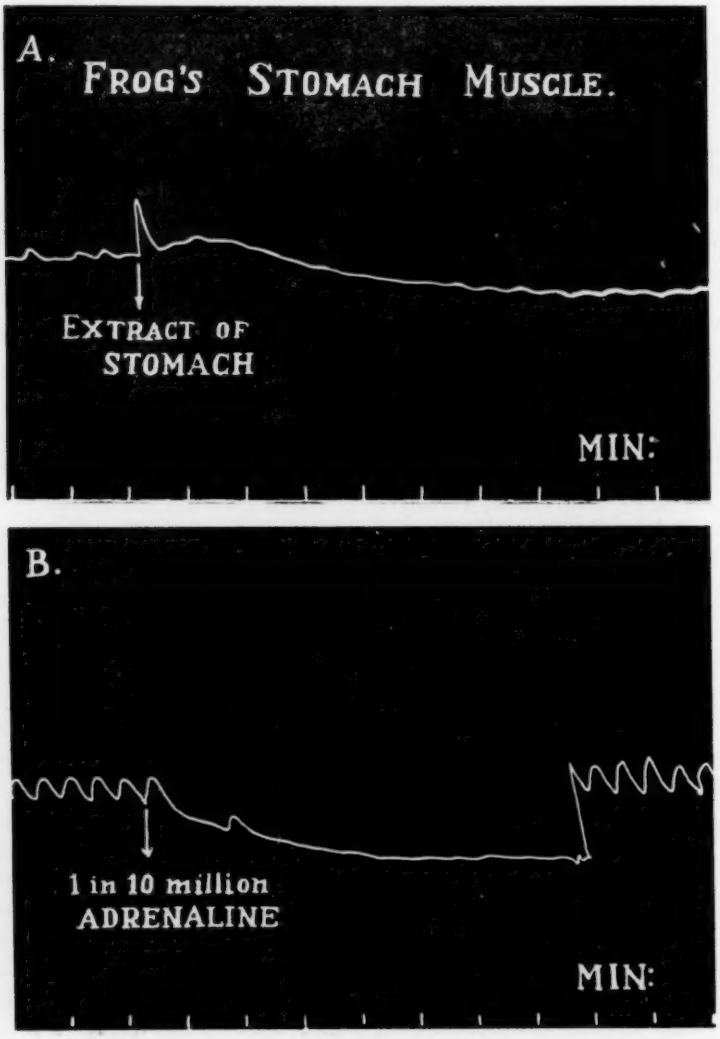

FIG. 1. Frog's stomach muscle. A. Effect of extract of stomach heated to 60° C. for one hour. B. Effect of 1 in 10 million adrenaline.

Just freezing the muscle for one hour and then thawing does not release any acetylcholine. This suggests that acetylcholine is not contained in the muscle fibres by the membrane, which would be destroyed on freezing and thawing. It must be then chemically bound and keeping temperature low, there is a chance of extracting the bound acetylecholine as such and knowing whether it is bound to the contractile mechanism.

The above experiments were performed on autumn frogs, room temperature 20–25° C. In winter the results were quite different (room temperature, 13–15° C.). At this temperature no acetylecholine diffused out of the muscle, but raising the temperature to even 20° C. caused liberation. This shows that if the muscle had acclimatised to low temperature, then acetylecholine is more easily liberated by a rise of temperature. Besides seasonal variations, individual variations may occur.

Discussion

The stomach muscle from *Rana tigrina* is remarkable in several ways. It reacts like a normal muscle and shows action potentials in the complete absence of any electrolyte in the external medium (Singh, and Bhatt, 1957; Singh and Acharya, 1957 *a*). It reacts in saline in which all the sodium had been replaced with potassium and can be excited in the absence of any ionic gradient of sodium or potassium across the membrane (Singh and Acharya, 1957 *b*). If glycerinated, it fails to react to adenosine triphosphate (Singh, 1958).

The acetylcholine in this muscle is intracellular as in nervous tissue and it is most likely that it has some important intracellular function to perform which may or may not have any relation with neuromuscular transmission. This intracellular function may be its chief function, and its leakage from the muscle cells into the surrounding fluid may be the " smoke coming out of the chimney ", and its role in neuromuscular transmission may be " the utilisation of a by-product".

If in muscle, these neurohormones are attached to the contractile mechanism, they may be playing an important role in contraction and relaxation. Acetylcholine appears to cause contraction and adrenaline, relaxation of the contractile mechanism (Singh and Singh, 1950). It is interesting to note that if the muscle is heated to 50–60° C., it relaxes actively, and acetylcholine leaks out. It might be that the dissociation of acetylcholine from the contractile mechanism leads to active relaxation, so that acetylcholine would be concerned with the contractility of muscle. This might explain the findings of Burn, that acetylcholine revives a heart which has stopped

beating, and of Chujyo (1952), who correlates the acetylcholine of intestines with peristalsis.

As this muscle contains two contractile systems, one of which relaxes passively, and the other actively (Singh and Acharya, 1958 *a*). it is possible that acetylcholine acts on the first system and adrenaline or noradrenaline on the second. One should therefore look for some complex between the actomyosin system and the neurohormones.

SUMMARY

1. Frog's stomach muscle when immersed in eserinised Ringer's solution for 2 hours shows no leakage of acetylcholine, whether extended or unextended.

2. Electrical stimulation of the muscle or its nerves does not release any acetylcholine.

3. Depolarisation with strong solutions of potassium chloride does not liberate acetylcholine.

4. Immersion in strong solutions of calcium chloride does not liberate acetylcholine.

5. Heating to 50–60° C., or treatment with ether liberates acetylcholine; the muscle also relaxes actively if heated. Adrenaline or noradrenaline is also liberated by heating.

6. As heating to 50–60° C. causes active relaxation as well as liberation of acetylcholine, it is suggested that acetylcholine is in some way connected with contractility by direct action on the contractile mechanism.

7. The above results were obtained in autumn, but in winter acetylecholine was liberated.

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A NEW SPECIES OF *MICROPHANURUS* KIEFFER

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***Microphanurus indicus* sp. nov.**

Female

Length.—1·1 mm. approx.

Colour.—Black. Legs, except coxæ and terminal tarsal segments, antenna except radicle and club bright honey-yellow; radicle and the last four antennal segments dark brown, almost black.

Head.—About one and one-third times wider than long, clearly wider than thorax. Frons slightly convex, central part smooth and shiny with transverse striations above the antennal insertions which are not contiguous; there being a mid-vertical ridge extending upto the anterior ocellus; this striated area not extending right up to the anterior ocellus. Vertex between the posterior ocelli roundly angled, without a trace of any ridge, with thimble-like punctures extending down to the sides of frons bordering the eyes and become feeble further down. Interocular space slightly less than one and a half times the interocellar space; the latter slightly over one and a half times the front ocellar and about five times the ocelocullar space. Mandibles brown, large and tridentate. Antennæ slightly pubescent; relative length of the antennal segments from radicle onwards as below: 8, 38, 12, 10, 6, 5, 4, 5, 6, 7, 7, 10. Antennal sockets of their own diameter apart.

Thorax.—Raised high above the level of abdomen but does not appear unusually large as in *M. painei* Ferrière. Mesonotum with a minute whitish hair projecting out of each such puncture. Scutum without any trace of parapsidal furrows, broader than long and twice the length of scutellum. Mesopleura sharply margined below in front; with a row of pits lining its margin and more so in the upper half leaving a small smooth shiny surface in the lower half. Metapleura also with such pits in its greater part; on its mid-upper half there is a small flat shining space.

Abdomen.—Scarcely longer than wide, 9:8. Tergite 2 in its greatest mid-part striated longitudinally almost to apex; striæ somewhat weak distally; sides smooth and shiny.

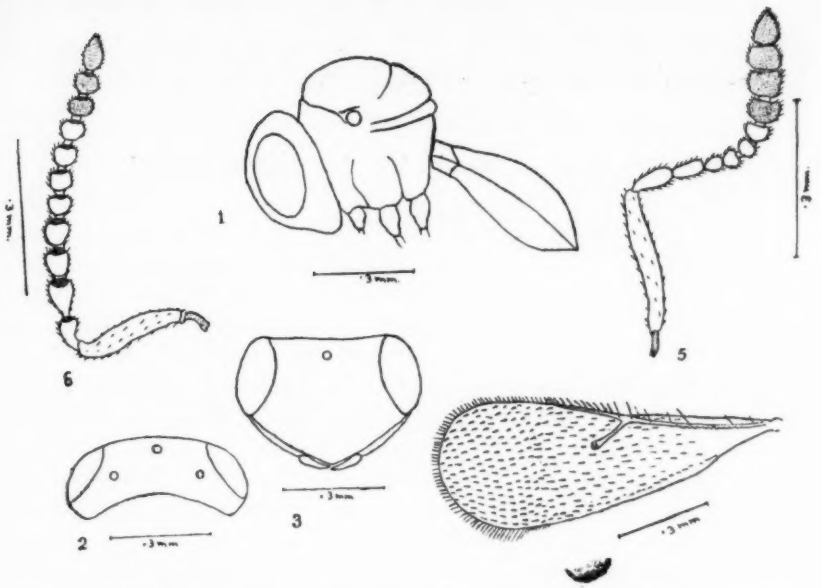

FIG. 1. 1. *Microphanurus indicus* Sp. nov. ♀ side view. 2. Vertex. 3. Head viewed from in front. 4. Forewing. 5. Antenna (female). 6. Antenna (male).

Wings.—Forewings thrice longer than broad; a little longer than the hind wing. Venation faint brown; stigmal about twice the marginal; hair of sub-marginal projecting well beyond the edge of the wing; marginal fringe short.

Legs.—Hind tibia a little longer than its femur and about one and a half times the length of foretibia; first tarsal segment less than the combined length of the rest.

Male

Similar to female except that of antennæ. Radicle dark brown, last three segments brown, rest honey-yellow. Radicle one fourth the length of scape. Pedicel one-thirds the length of scape and a little smaller than the first flagellar segment. Segment 6–11 are all transverse and almost equal. *Microphanurus indicus* sp. nov. runs close to *M. painei* Ferrière but differs from it chiefly in the ratio of antennal segments, sculpturing of mesopleura and striations of the abdomen.

Described from 6 females and 1 male,

Holo and Allotypes deposited in National Pusa Collection, Indian Agricultural Research Institute, New Delhi.

Collected.—R. B. Kaur, 7th April 1958.

Type locality.—New Delhi.

Host.—Eggs of a pentatomid bug *Plautia fimbriata* Fabr.

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HOST SELECTION AND OVIPOSITION RESPONSE IN *APANTELES ANGALETI* MUESEBECK (BRACONIDÆ: HYMENOPTERA)

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Introduction

Apanteles angaleti Muesebeck is a solitary endoparasite of the pink bollworm *Platyedra gossypiella* Saunders in India. The parasite was first reared from infested bolls collected from experimental cotton fields of the Agronomy Division in the parasite laboratories of the Indian Agricultural Research Institute during the summer months of August and September 1954 and it was described as a new species to science by Muesebeck (1954). The parasite is being bred in large numbers on the alternate host *Corcyra cephalonica* Stainton in the parasite laboratories of the Indian Agricultural Research Institute by adopting the technique described by Narayanan *et al.* (1956). *Platyedra gossypiella* is a pest of world importance and causes severe damage to cotton bolls not only in India but also in the other major cotton-growing countries of the world, like Pakistan, Sudan, Egypt and U.S.A. The infestation sometimes is so serious that entire cotton fields are almost devastated. So the control of the pest assumes particular significance especially in relation to the rural economy in the cotton-growing areas of the Indian Union. Yet, the control of the pest is one of the baffling problems that has ever faced the economic entomologists, not only in India but also in other countries of the world where cotton is grown. The insecticidal method of control though practised in the most advanced countries of the west, notably the United States of America, has not effectively solved the problem. So the U.S. Department of Agriculture have sent their entomologists from time to time on missions to comb the other cotton-growing areas of the world, to discover new natural enemies of the pest with a view to introduce and establish them in the new world to control the pest. For, after all, the insecticidal method of control is only palliative that has to be repeated year after year. More fundamental in approach is the biological basis of the insect control. *Apanteles angaleti* is one of the primary parasites that can be utilized in the control of this serious pest. But before any parasite can be released in the

field it is of utmost importance that its biology and ecology should be studied in detail in the laboratory. Biology and ecology are the basis of all insect control. In fact, a good deal of work has been done on the host selection of ectoparasites but the studies on the endoparasites seem to have been neglected to a great extent. We have very few published literature on this subject. Salt (1935) has made an exhaustive study of host selection in *Trichogramma evanescens* Riley. But he as well as others have studied host selection by utilizing a number of different species of hosts, whereas the present studies are confined only to the selection of a suitable developmental stage of the same host, *Corcyra cephalonica* Stainton. In this paper the host selection and oviposition response in *A. angaleti* has been studied in detail.

MATERIAL AND METHODS

Corcyra eggs were kept in separate bottles every day for hatching and the bottles were numbered and particulars about the date on which the eggs were put into the bottles and the date on which they hatched were clearly written on the bottle by means of a coloured glass pencil. Freshly hatched larvæ were taken out by means of a fine camel hair-brush and a known number of larvæ were placed in a small petri dish containing a small quantity of fine flour (Maida). The petri dish was covered with a lid and held tight by means of India rubber bands. Three such dishes were prepared for each experiment each day and kept in a desiccator maintaining 70% relative humidity and the latter placed in an incubator running at constant temperature of $27 \pm 2°$ C.

Each of these petri dishes containing the required number of known aged larvæ was exposed to a pair of freshly emerged mated female parasites and a piece of raisin was given as food. The petri dishes containing the host larvæ and the parasites were placed back in the desiccator. After every 24 hours the dishes were removed and the parasites were carefully transferred to another set of dishes containing unparasitised host larvæ of the same age. This was continued until the parasites died. Every day the host larvæ were dissected to find out the number of eggs contained in each host larva. Knowing the age of the host larvæ, the number of hosts dissected, the number of hosts parasitised and the number of eggs laid, the percentage of parasitism was calculated. Olfactometer experiments were also performed to corroborate the results obtained by the dissection method.

OBSERVATIONS

The females after being confined to oviposition cages immediately start searching for the hosts if they have completed their pre-oviposition period,

which is usually 24 hours in this species, by vibrating their antennæ on the thinly spread flour above the host larvæ. After the host has been located, the selection of a suitable host is done by means of her ovipositor which can be observed piercing through the flour to reach the host larvæ.

The experiments were started with 24 hours old larvæ and were repeated with the increase in age by every 24 hours. During the course of these investigations, larvæ from one to eighteen days old were offered to the parasite to study the oviposition response.

The results obtained in the experiments on the dissected host larvæ are tabulated in Table I.

TABLE I

Showing the oviposition response of Apanteles angaleti *to different ages of the host larvæ*

No. of Exp.	Age of the host	No. of Dissections	Total No. of larvæ parasitised	Total No. of eggs laid	Maximum No. of eggs laid in a single host	Percentage of eggs laid	Percentage of parasitism
1	24 hours	139	4	4	1	2·87	3
2	48 hours	124	11	16	3	12·9	9
3	72 hours	674	240	306	3	45·7	35·6
4	96 hours	364	168	354	11	97·20	46·15
5	120 hours	314	189	314	8	100	60·2
6	144 hours	248	180	277	**13**	**111·7**	**72·58**
7	168 hours	600	364	670	7	111·66	60·66
8	192 hours	467	200	342	10	73·34	42·82
9	216 hours	415	58	102	9	24·57	13·97
10	240 hours	252	23	34	4	13·5	9·1
11	264 hours	249	16	24	2	9·6	6·4
12	288 hours	140	..	..	..	..	..

It is quite clear from Table I that oviposition response is maximum when 6 days old larvæ are exposed, though there is not much significant difference among the 5, 6 and 7 days old larvæ. It is interesting also to observe that all these stages are of the same instar. The percentage of parasitism is 60·2, 72·58 and 60·66 respectively for the three different ages. Preference to 6 days old larvæ is also clearly indicated by the

maximum number of eggs laid, *i.e.*, 13 in a single host. After this age the oviposition response slowly decreases and when the host is of 12 days old hardly any egg is deposited.

Table I is graphically represented in Fig. 1. The curve shows a gradual rise in the percentage of parasitism from one day old larvæ to six days old larvæ, which is the peak of the curve and is therefore the optimum age of the larvæ suitable for oviposition. From 6 days onwards the percentage gradually falls down somewhat in the same way as it had risen in the beginning of the curve.

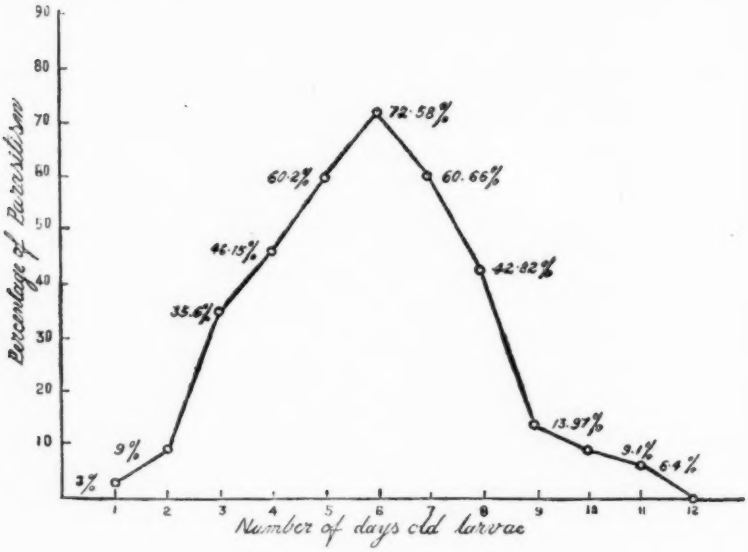

FIG. 1

After completing the experiments on host selection behaviour of *Apanteles angaleti* a few experiments were also conducted in a modified McIndo's Olfactometer to investigate the olfactory responses of the parasite to various stages of host larvæ. The Olfactometer experiments were conducted mainly to corroborate the earlier results obtained and also to find out whether the parasites could select the host through olfactory senses. Host larvæ of different ages were used for experiments and the following results have been obtained:

Oviposition response of Apanteles angaleti *to different ages of* Corcyra *larvæ in Olfactometer*

EXPERIMENT I

Sl. No. of Experiment	No. of females entered the arm of Olfactometer containing	
	1 day old larvæ	5 days old larvæ
(*a*)	5	10
(*b*)	6	6
(*c*)	4	6
(*d*)	10	4
(*e*)	3	2
TOTAL ..	28	28

EXPERIMENT II

Sl. No. of Experiment	No. of females entered the arm of Olfactometer containing	
	5 days old larvæ	9 days old larvæ
(*a*)	3	12
(*b*)	11	7
(*c*)	9	2
TOTAL ..	23	21

EXPERIMENT III

Sl. No. of Experiment	No. of females entered the arm of Olfactometer containing	
	6 days old larvæ	12 days old larvæ
(*a*)	6	7
(*b*)	6	7
(*c*)	5	2
TOTAL ..	17	16

EXPERIMENT IV

Sl. No. of Experiment	No. of females entered the arm of Olfactometer containing	
	5 days old larvæ	19 days old larvæ
(*a*)	7	9
(*b*)	4	12
(*c*)	3	12
(*d*)	1	8
TOTAL ..	15	41

The results of the above experiments are not very significant.

It is seen that irrespective of the age of the host larva the ovipositing females entered one or the other arm of the Olfactometer containing host larvæ. It is evident that the females are attracted only to the smell emitted by the host larvæ, but they are not able to discriminate further the age of the host larvæ by different smells or in other words there is no difference in the odour emitted by the larvæ of different ages.

DISCUSSION

Notwithstanding the mass of published literature that exists relative to the hosts of entomophagous parasites, we are completely in the dark as to the determining factors that govern certain parasites constantly to select certain host species, while others utilise hosts of different taxonomic relationships. The vast majority of parasites are but seldom restricted to a single host species. *Apanteles angaleti* is an endoparasite of *Platyedra gossypiella* Saunders in India which has not been recorded on any other host hitherto.

When insects reproduce in artificial confinement, the need for utilising the sense of smell for finding out their mates, food or egg deposition sites is generally diminished. Nevertheless, it has been observed that odour may be a critical factor in the mass production of certain species of parasitic Hymenoptera (Flanders, 1944). During the course of the present investigations on the host selection of *A. angaleti*, it has been observed that the faculty of chemical senses is not at all utilised. In this particular case the investigations were confined to the same species of host namely *Corcyra cephalonica* St.,

but to different ages of the host larvæ. It was observed that when larvæ were kept for development for about 12 days in small quantities of fine flour (Maida) some developed better than the others, in spite of the same ecological environments. When such a group of larvæ, though of the same age but of different growths were offered for parasitisation, the female parasites preferred the smaller ones. On the other hand, newly hatched larvæ were not preferred by the parasite. The percentage of parasitism slowly rises to the maximum when the host larvæ of about 6 days old are offered. From this period onwards the percentage of parasitism decreases and in 12 days old larvæ very few eggs are laid. From this experiment we can safely conclude that the parasite prefers larvæ that are 5, 6 or 7 days old and rejects freshly hatched or 12 days old larvæ. When too small or too big hosts are offered, the parasites tend to retain their eggs rather than deposit them and if a few eggs are laid, it is only due to the restraint on the part of the female parasites.

Salt (1935) made an attempt to discover by well planned scientific experiments and to express in precise scientific language some of the criteria by which ovipositing females of *Trichogramma evanescens* Riley, choose their hosts. The criteria which he examined were: (1) position, (2) odour, (3) texture, (4) colour, (5) absolute size, (6) shape. After examining all these criteria, the author came to certain definite conclusions. For instance, he defined the objects accepted as "hosts" and one on which the fertile offsprings can generally be reproduced as "suitable hosts". Taking this as an example, it can safely be said that the suitable stage of host of *Apanteles angaleti* in mass breeding is 4 to 8 days old *Corcyra* larvæ with the maximum reproduction in 6 days old larvæ.

It has been observed that *A. angaleti* is unable to distinguish a healthy host from that of a parasitised one and this results in superparasitism. Eggs are laid under darkness as well as light and a maximum of 13 eggs have been dissected from a single host. The female parasite in the site of the host larvæ goes on vibrating her antennæ to locate the host (which is imbedded in the flour) and once the host is located by means of her olfactory senses, the selection of a suitable host is done by means of her ovipositor. Hence it is not necessary that every time the ovipositor is seen piercing the host body, an egg should be deposited. In fact most of attempts fail if the texture of the larval integument is too tough for piercing. It is evident that oviposition is guided by a stimulus which is not chemical but physical namely the sense of touch. It can be stated with certainty that the selection of a suitable stage of host is dependent on its physical characters such as size or developmental condition and rigidity or hardness of the host integument.

A series of Olfactometer experiments conducted to find out the olfactory response of the female parasites to various stages of the host showed that the parasites could not distinguish between the two developmental stages of the host. Had selection been dependent upon odour, the majority of females should have shown preference to a suitable stage of the host larvæ but the results were most inconsistent and the preference to hosts based upon odour was not in agreement with the actual oviposition response experiments. There can be little doubt that the odour emitted by various stages of the *Corcyra* larvæ is more or less the same. Hence the parasite was only able to locate the host with the sense of smell but was not able to oviposit due to the lack of oviposition stimulus.

Most of the earlier workers have emphasised upon the chemical senses as the basis of the host selection. But their work concerns mostly with entomophagous parasites selecting a suitable host from different species. However, there is no literature on host selection of an endoparasite selecting a suitable host from the same species but of different developmental stages. So, the present studies add to our knowledge of the fundamental aspects of parasite specificity. The selection in case of *Apanteles angaleti* is based primarily upon the physical characters of the host larvæ and the stimulus for oviposition is the sense of touch and not that of smell which is often seen in most of the other entomophagous Hymenopterous parasites.

SUMMARY

1. *Apanteles angaleti* Muesebeck is a solitary endoparasite of the *Pink* bollworm, *Platyedra gossypiella* Saunders in India.

2. The host selection of the parasite has been studied in detail. The females prefer 4 to 8 days old larvæ for oviposition and the maximum eggs are deposited in 6 days old larvæ.

3. Various factors which stimulate oviposition in parasites in general and particularly in the case of *Apanteles angaleti* have been discussed. In this case the host selection is based primarily on physical characters of the host larvæ such as rigidity of integument. Stimulus for oviposition is the sense of touch and not that of smell.

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LABELLING OF ADULTS OF AN INSECT PARASITE *BRACON GELECHIÆ* ASHMEAD WITH RADIOACTIVE PHOSPHORUS (P^{32})

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Introduction

With a view to study the distribution of insects under field conditions, their flight range, the capacity of the parasites to locate their hosts, etc., the insects were used to be labelled with coloured dyes. The use of radioactive isotopes revolutionised the method of labelling insects. A number of methods of labelling insects with radioactive isotopes have been described by some workers within recent years which to some extent have surpassed in efficiency the earlier methods of labelling insects with coloured dyes. Roth and Hoffman (1952) made flies and mosquitoes radioactive by dipping them individually for ten seconds in P^{32} labelled phosphoric acid solution after immobilising them with carbon dioxide. This method is quite a complicated and laborious one, especially when quite a large number of labelled insects are required for mass release in the field. Furthermore, conventional methods of labelling insects with radioactive isotopes by physical application to the body of the insect are equally impracticable in the case of tiny and delicate parasitic insects. Lindquist, Yates, Hoffman and Butts (1951) demonstrated that the adults of *Musca domestica* Linn., *Lucilia sericata* Meig., and *Phormia regina* Meig., could be made radioactive by confining them with water containing radioactive phosphoric acid. A slightly modified version of this method, *i.e.*, glucose solution made radioactive with a solution of P^{32} labelled phosphoric acid was used and it was found that it did not work well with the adults of the parasite, *Bracon gelechiæ* Ashmead, as they were unable to pick up enough radioactivity. Yates, Gjullin, Lindquist and Butts (1951) produced radioactive adults of *Aedes* sp. by allowing them to feed on a rat that had been given previously an intraperitoneal injection of radioactive phosphoric acid. Jensen and Fay (1951) made adults of *Musca domestica*, *Callitroga macellaria* and *Phænicia pallescens* radioactive by rearing them in a medium containing radioactive phosphoric acid. The latter method appears to be promising which was modified in this laboratory to

suit the requirements of *Bracon gelechiæ*, a beneficial larval parasite of a serious pest of potatoes *Gnorimoschema operculella* Zell.

MATERIALS AND METHODS

Phosphoric acid (H_3PO_4) labelled with P^{32} having a specific activity of 0·5 mc. per c.c. which was supplied by The Radiochemical Centre, Amersham, England, was used. The experiments were conducted with the radioactive phosphoric acid after making the necessary dilutions with distilled water. The actual details of the procedure are given along with the experiments.

The measurements on the activity of the adult parasites and their host caterpillars were taken with the help of a halogen quenched, end-window mica, G. M. tube and a scaling unit supplied by Philips India Ltd. The mica thickness of the G.M. tube window was 2–3 mg./cm.^{2} To reduce the error in counting rates due to the background counts, the G.M. tube as well as the sample were housed in a convenient box prepared out of 3/16 inch thick perpex. Under these shielding conditions the background count rate was 30 ± 5 counts per minute. During all the measurements on activity of the insects, a fixed geometry was maintained. The adult parasites as well as their host caterpillars were kept singly in one inch diameter aluminium flat planchet and positioned in the G.M. tube assembly so that the adults were 3 cm. away from the G.M. tube window.

B. gelechiæ and *Corcyra cephalonica* Stainton used in these experiments were obtained from the cultures maintained in the divisional laboratory. The eggs of *C. cephalonica* were obtained by keeping gravid females in tin oviposition cages having glass top and wiregauge bottom. These containers were placed in trays that were to receive the eggs laid by the moths. The females oviposited through the wiregauge and the eggs dropped in the trays below were collected. The larvæ hatching from these eggs were kept in glass jars covered with muslin at the open end and they were fed on crushed maize. *B. gelechiæ* was conveniently reared as follows: The males and females were kept in 3×2 inches glass dishes containing cotton pads soaked in 10 per cent. glucose solution and covered on one end with muslin, the full-grown host caterpillars of *C. cephalonica* being kept on the muslin and covered over by the glass plate. The female parasites paralysed the host caterpillars and laid eggs on their body which hatched and the young grubs fed on the tissue of the host. Pupation took place within 48–72 hours after hatching and the adults emerged about a week thereafter. All these insects were reared at $25 \pm 2°$ C. and 70 ± 4 per cent. relative humidity.

Experimental Details and Observations

The adults of *B. gelechiæ* were labelled by feeding them on 10 per cent. glucose solution containing different activities of radioactive phosphoric acid. Dilutions of the radioactive phosphoric acid were made with 10 per cent. glucose solution in a manner that gave 25, 50 and 100 microcuries of the radioactivity per c.c. Small cotton pads were soaked with 1 c.c. of different dilutions of the radioactive material and kept in separate rearing jars along with the single mated female parasite, the mouth of the jar being covered with a fine muslin cloth held in position by Indian rubber bands. To prevent external radioactive contamination of adult insects from direct contact with the cotton pads, the latter were covered with a wiregauge of fine mesh. Simultaneously a control with 10 per cent. glucose solution only as feed was also kept. All the cages were kept in a chamber at 72–92° F., with 62-87 per cent. relative humidity. Each experiment along with the control was replicated four times. After feeding the adults for 24 hours both the living and the dead ones were removed from the cages and assayed for radioactivity. The dead ones were assayed as such, whereas the living ones were anæsthetised with chloroform and then assayed. Observations on their activity measurements are given in Table I.

Table I

Treatment	Average activity in c.p.m. of the adult parasites	Percentage mortality after 48 hours
25 μc. of P^{32} ..	30	100
50 μc. of P^{32} ..	70	100
100 μc. of P^{32} ..	120	100
Control ..	nil	50

It is evident that there is very heavy mortality after 48 hours among the parasites fed on radioactive phosphoric acid of 25–100 μc. per c.c. of 10 per cent. glucose solution. Moreover, in view of the very low activities shown by the adults fed on the lowest dose of the active material in these treatments it is not practicable to get adults of still lower activity with further reduced dosages of radioactive substance in the food, which could be easily assayed.

The main idea in these experiments is to get labelled adult parasites which are sufficiently radioactive and which could survive for a considerable time with enough detectable radioactivity so that they could be tried for mass release under field conditions. The main purpose is not served by this method of labelling the parasites. Consequently the technique of Jensen and Fay (1951) was modified to suit the parasite. The technique is described below:

The parasites were reared on host caterpillars which were made radioactive by allowing them to feed on food mixed with radioactive phosphoric acid. Radioactive phosphoric acid of 500 and 750 microcuries activity was mixed with 30 grams of crushed maize and to facilitate uniform mixing of the active substance a little quantity of distilled water was added. The ingredients were thoroughly mixed and dried under an infra-red lamp. Thirty, fifth and sixth instar caterpillars of *C. cephalonica* which were previously starved for 48 hours were released in each lot of maize and allowed to feed for 48 hours. The caterpillars were then washed thoroughly with water to remove radioactive contamination from external parts of their body. The repeated washing of these caterpillars showed that a minimum of four thorough washings were sufficient to decontaminate the body surface completely. The caterpillars were then dried over filter-papers and assayed in shallow aluminium planchet with the same geometry and under the same G.M. tube as was used in the previous experiment. While assaying, the caterpillars were made to remain stationary in the planchet by covering it with a thin cellophane paper. The absorption of radiations by the cellophane covering was negligible. The active caterpillars were separated in three activity ranges (*viz.*, 100–250 c.p.m., 251–500 c.p.m. and 501–1000 c.p.m.) and were given to the mated females of *B. gelechiæ* for parasitisation. The parasitised caterpillars along with the parasite eggs on them were removed and kept in 4 inches diameter petri dishes for further development at 72–92° F. and 62–87 per cent. relative humidity. The parasite grubs after hatching continued feeding on the host caterpillars, they later pupated and eventually emerged as adults. Observations on the developmental period from the egg to the adult on radioactive host caterpillars and the longevity of the emerging adults are given in Table II. Some of these adults were collected separately in test-tubes, anæsthetised with chloroform and assayed for radioactivity. The measurements on the radioactivity of the adult parasites are given in Table II.

The adult parasites reared on the radioactive host caterpillars of *C. cephalonica* contained enough detectable radioactivity. Further the

TABLE II

Range of activity in c.p.m. of host caterpillars	Average activity in c.p.m. of the host caterpillars exposed for parasitisation	Temperature °F.	Per cent. relative humidity	Time required to complete development of parasite in days	Longevity of adult parasites in days	Average activity in c.p.m. of freshly emerged adult parasites	
						Male	Female
100– 250 ..	192	72·92	62·87	10·12	6·9	60	75
251– 500 ..	408	72·92	62·87	10·12	6·9	98	157
501–1000 ..	677	72·92	62·87	10·12	4·9	176	287
Control ..	Without any activity	72·92	62·87	10·12	6·8	..	..

radioactive adults lived for a period which is sufficient for the purposes of mass release with a view to study population density, flight range, etc. Moreover, it may be mentioned that the developmental period and the longevity of the adults were not adversely affected by these levels of radioactivity. The labelling of the adults of *B. gelechiæ* by rearing them on radioactive host caterpillars appears to be a more effective method than allowing the adult parasites to feed on glucose solution containing radioactive phosphoric acid. It appears that the rearing of parasites on host caterpillars having an average activity of 677 c.p.m. is most suitable to obtain the labelled adult individuals. Incidentally the adult female parasite reared in this way show more radioactivity than the males. This is an important point as it is the female which after fertilisation has to go in search of the host for parasitisation. In view of this there is greater necessity of finding out the flight range, dispersal, population density, etc., of the female and consequently if they are labelled with tolerably higher radioactivity it is easier to detect them even after a week of the initial mass release when the activity of the P^{32} in their body after going through some radioactive decay and physiological elimination is still enough for the purposes of easy detection.

SUMMARY

Labelling of the adults of *Bracon gelechiæ* Ashmead by allowing them to feed on 10 per cent. glucose solution containing phosphoric acid (H_3PO_4) with P^{32} is not practicable as the adult parasites are unable to pick up enough radioactivity. On the other hand labelling of the adults by rearing them on host caterpillars of *Corcyra cephalonica* Stainton, the latter being fed on crushed maize mixed with radioactive phosphoric acid is quite a convenient and satisfactory method for the purpose of mass release of the parasite under the natural conditions, as such individuals show tolerably high degree of radioactivity and are therefore easy to detect. Moreover, the developmental period of the parasite by rearing it on radioactive host caterpillars is not adversely affected and the adults remain alive for a considerable period.

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STUDIES ON *RHIZOBIUM* FROM *ZORNIA DIPHYLLA*

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Root nodule bacteria from a large number of leguminous plants have been reported in the literature. These organisms are able to symbiose with certain plants other than their original host plants. But their ability to do so is restricted within certain group of plants only, which is an important criterion in the classification of rhizobia. Whereas Fred *et al.* (1932) could describe 15 bacterial plant-cross-inoculation groups, Wilson and Sarles (1939) at a late stage have been able to describe 21 such groups. The Bergey's Manual has recognized, however, only six species; the species for all other groups is still in question. Nowhere in the literature the nodule bacteria from *Zornia diphylla* have been reported so far.

Zornia diphylla is a weed growing abundantly in the Charotar area of Gujarat. The leaves are small like grass and bears small yellow leguminous flowers. The plant grows abundantly in monsoon.

Materials and Methods

Isolation of rhizobia from the root nodules.—The plants were carefully pulled out and roots thoroughly washed with water. Some healthy and intact nodules were selected for the bacteriological work. Isolation of bacteria from the nodules was done by the usual methods, on yeast extract mannitol congo-red (1: 20,000) and crystal violet (1: 80,000) agar. All the isolates were then tested for the absence of *A. radiobacter*, on litmus milk, plain milk, potato plants, yeast extract mannitol broth pH 11–12, yeast extract mineral salts medium with $CaCO_3$ *plus* (*a*) 1% dextrin, (*b*) 1% inulin, Koser's uric acid medium, mannitol calcium glycerophosphate agar, crystal violet glycerol nitrate soil extract agar, and for production of H_2S by ZoBell's method (1950). All the isolates were found to be free from *A. radiobacter*.

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2. The cultural, physiological and biological properties of the isolate were studied under the following heads:

(*a*) Reaction change in litmus milk and bromthymol blue milk.

(*b*) Fermentation of carbohydrates, alcohols and organic acid salts.

(*c*) Liquefaction of gelatin.

(*d*) Test for tryosinase activity.

(*e*) Production of indol.

(*f*) Reduction of nitrates.

(*g*) Reaction tolerance.

(*h*) Thermal death point.

(*i*) Symbiotic study with the host slant.

Litmus milk and bromthymol blue milk were prepared and sterilized following the methods recommended by *Manual of Methods for Pure Culture Study of Bacteria* (1948–49). The tubes were incubated at 28° C. for 15 days.

The basal medium for the study of fermentation of carbohydrates, alcohols and organic acid salts consisted of mineral salts and yeast extract (Difco) similar to one used by Georgi and Ettinger (1941), the fermentation of the carbohydrate and bromthymol blue as an indicator. The change in reaction as indicated by the indicator was observed for a week and reported.

The tyrosinase activity was tested in asparagine mannitol nitrate medium containing 0·15% tyrosine. The production of indol was studied in a medium containing 1% tryptone, 0·5% glucose, 0·005% Bacto yeast extract and mineral salts. The test for indol was done, when good growth was visible, usually after four days.

The test for H_2S was done in a medium containing 1% tryptone, 1% mannitol and 0·005% Bacto yeast extract, by adopting ZoBell's lead acetate test strip method. The reduction of nitrates was also studied in mannitol nitrate medium containing 0·005% Bacto yeast extract. The similar yeast extract mannitol medium was adjusted to different reactions to study the reaction tolerance of the organisms. The thermal death point of the isolate was determined in the same medium contained in $5'' \times \frac{5}{8}''$ test-tubes.

The host plants were raised under aseptic conditions in test-tubes ($8'' \times 1''$), containing sterile sandy loam soil, to study the symbiotic properties of the isolates.

RESULTS AND DISCUSSION

TABLE I

Growth characters of the Rhizobium *in different media for differentiation from* A. radiobacter

Sl. No.	Name of the medium	Growth characters of the *Rhizobium*
1	Plain milk	Serum zone present
2	Litmus milk	Alkaline, Serum zone present
3	Potato slants	Slight growth only
4	Yeast extract mineral salts with $CaCO_3$ *plus*	
	(*a*) 1% dextrin	Alkaline reaction
	(*b*) 1% inulin	Alkaline reaction
5	Yeast extract mannitol broth:	
	(*a*) pH 10	No growth
	(*b*) pH 11–12	No growth
6	Koser's uric acid medium	Slight growth only
7	Mannitol calcium glycerophosphate agar ..	Raised slimy growth without browning of the medium
8	Crystal violet glycerol nitrate soil extract agar	Small evenly coloured colonies
9	Production of H_2S	Not produced

TABLE II

Cultural, physiological and biological characters of the Rhizobium

Sl. No.	Name of the reaction Nature of the test	Reaction exhibited by the *Rhizobium*
1	Reaction change in litmus milk and bromthymol blue milk	Alkaline reaction with the formation of serum zone of 0·5 mm. height
2	Liquefaction of gelatin	Not liquified even after incubation for two months
3	Tyrosinase activity	Absent
4	Production of indol	Absent
5	Reduction of nitrates	Nitrates reduced to nitrites
6	Reaction tolerance in yeast extract mannitol medium	pH 5·6 on acid side. pH 8·6 on alkaline side
7	Thermal death point in yeast extract mannitol medium	58° C.

TABLE III

Growth and fermentation characters of the Rhizobium *in different carbohydrates*

Sl. No.	Carbohydrate	Growth of the *Rhizobium*	Reaction
1	Rhamnose	Fair	*a*
2	Arabinose	Fair	*aa*
3	Xylose	Good	0
4	Glucose	Good	*aaa*
5	Galactose	Good	*aaa*
6	Mannose	Fair	*a*
7	Sucrose	Fair	*b*
8	Lactose	Fair	*b*
9	Maltose	Good	*bb*
10	Raffinose	Fair	*bb*
11	Dextrin	Poor	*b*
12	Inulin	Fair	*bb*
13	Starch	Poor	*bb*

a = Slight acidity.
aa = moderate acidity.
aaa = Strong acidity.
0 = No change in reaction.
b = Slight alkaline.
bb = Moderately alkaline.
bbb = Strongly alkaline.

TABLE IV

Growth and fermentation characters of the Rhizobium *in alcohols and organic acid salts*

Sl. No.	Alcohol or organic acid salt	Growth of the *Rhizobium*	Reaction
1	Ethyl alcohol	Fair	*bb*
2	Glycerol	Good	*bb*
3	Mannitol	Good	*aa*
4	Dulcitol	Poor	0
5	Sodium tartrate	Fair	*bbb*
6	Sodium succinate	Fair	*bb*
7	Sodium lactate	Fair	*bb*
8	Sodium citrate	Poor	0
9	Sodium formate	Poor	0

a, aa, aaa, b, bb, bbb and 0—same as in Table III.

The *Rhizobium* was found to be able to produce nodules on the pea plants. While it failed to nodulate cowpea, ' guwar ' (*Cyamopsis tetragonoloba*, Taub.), bean, lucerne (*Medicago sativa* L.), and berseem (*Trifolium alexandrinum*).

Morphologically the organisms are gram negative, motile with peritrichous flagella. In old cultures the bacillary to coccobacillary forms were common. Coccoid forms were abundant in one month old cultures. Bacteroids in the nodules were club-shaped, bulged at one end and few branched forms like Y.

DISCUSSION

From the very fact that the *Rhizobium* from *Zornia diphylla* is able to produce nodules on pea plants only, one may be inclined to include these organisms in pea cross-inoculation group of rhizobia. Its cultural reactions for differentiation of rhizobia from radiobacter are typical of rhizobia, in all the media tested without exception. All these reactions are also similar to those given by pea rhizobia (Vyas, 1955). It gives alkaline reaction with serum zone formation in litmus milk and bromthymol blue milk. The fermentation characters in different carbohydrates, alcohols and salts of organic acids are very much similar to those given by pea bacteria. It is gelatinase and tyrosinase negative and gave negative test for indol and H_2S. It reduces nitrates to nitrites. The pea rhizobia also give similar reactions (1955). The limit of acidity for the *Rhizobium* from *Z. diphylla* is pH 5·6, and limit of alkalinity pH 8·6, while the similar values for pea rhizobia are pH 5·6 and pH 8·4 respectively (1955).

All these reactions indicate close similarity between rhizobia from pea and from *Zornia diphylla*. This is suggestive of inclusion of this *Rhizobium* in pea cross-inoculation group of rhizobia.

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THE MYXOPHYCEÆ OF THE KUMAON HILLS, U.P., INDIA—I

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Bhâradwâja[1], Rao[2, 3] and Singh[4] have recorded in a series of papers the myxophyceous flora of several districts in the plains of Uttar Pradesh, but no serious efforts have so far been made to record these algæ from the hilly districts of Kumaon region. It is desired, therefore, to record the whole Myxophyceæ of this region in parts, and the present communication relates to the Myxophyceæ of Naini Tal* only. In all thirty-six forms have been described and most of them have been collected on the moist soil and rocks

Systematic Enumeration of the Species Observed

Myxophyceæ

1. *Aphanocapsa grevillei* (Hass.) Rabenh. Geitler, in Rabenhorst's *Kryptogamen-Flora von Europa*, Band XIV, *Cyanophyceæ*, 1930–32, p. 158, Fig. 71.

   Lat. cell., 3·5–4·5 μ.

   Habitat.—On moist soil, September 1956, Naini Tal.

2. *Microcystis æruginosa* Kütz. Geitler, *op. cit.*, 1930–32, p. 136, Fig. 59 *d*.

   Lat. cell., 2·5–3·5 μ.

   Habitat.—In Naini Tal Lake, along with other algæ.

3. *Aphanothece castagnei* (Breb.) Rabenh. Geitler, *op. cit.*, 1930–32, p. 171.

   Lat. cell., 3·9 μ; long. cell., 3·9–7·8 μ.

   Habitat.—On moist soil, Naini Tal.

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* Naini Tal is situated in latitude 29° 24′ North and longitude 79° 28′ East and at a height of 6,350 feet above mean sea-level. The climate on the whole is average. The temperature rises up to 90° F. in summer while in coldest months (December and January) it is an average of about 32° F., although temperature as low as 26° F. has been recorded. June, July, August and September are the chief rainy months and the average rainfall so far recorded is about 110″.

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4. *Glœcapsa montana* Kütz. Geitler, *op. cit.*, 1930–32, p. 186, Fig. 83 *d*.

   Lat. cell., 1·9–4·8 μ; long. cell., 5·8–7·8 μ; lat. colon. *cum* vag., 14·6–23·4 μ; long. colon. *cum* vag., 23·4–39·0 μ.

   Habitat.—On moist soil along with *Scytonema mirabile*, Naini Tal.

5. *Chroococcus minutus* (Kütz.) Näg. Geitler, *op. cit.*, 1930–32, p. 232, Figs. 112 *a* and 113 *c*.

   Lat. cell., 3·9–5·9 μ; long. cell., 3·9–6·0 μ; lat. cell. *cum*. vag., 6·8–8·5 μ; long. cell. *cum* vag., 8·5–10·2 μ.

   Habitat.—On moist rock along with *Nostoc linckia*, Naini Tal.

6. *Merismopedia glauca* (Ehrenb.) Näg. Geitler, *op. cit.*, 1930–32, p. 264, Fig. 129 *d*.

   Lat. cell., 3·5–6·5 μ.

   Habitat.—Planktonic in the fountain tank, Balrampur House, Naini Tal.

7. *Synechococcus cedrorum* Sav. Geitler, *op. cit.*, 1930–32, p. 273.

   Lat. cell., 3·9–4·2 μ; long. cell., 5·9–8·5 μ.

   Habitat.—On moist rock along with *Nostoc linckia* and *Chroococcus minutus*, Naini Tal.

8. *Oscillatoria sancta* Kütz. Geitler, *op. cit.*, 1930–32, p. 943, Fig. 598 *c*.

   Lat. trich., 11·7–15·6 μ; long. cell., 3·9–5·8 μ.

   Habitat.—From a temporary puddle, Naini Tal.

9. *Oscillatoria subbrevis* Schmidle. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *b*.

   Lat. cell., 5·1–6·8 μ; long. cell., 1·7–2·5 μ.

   Habitat.—On submerged stones, Sadiya Tal.

10. *Oscillatoria terebriformis* Ag. Geitler, *op. cit.*, 1930–32, p. 954, Fig. 607 *d*.

    Lat. cell., 4·2–5·1 μ; long. cell., 2·5–5·1 μ.

    Habitat.—On submerged stones, Sadiya Tal.

11. *Oscillatoria acuminata* Gom. Geitler, *op. cit.*, 1930–32, p. 978.

    Lat. cell., 3·4–5·0 μ; long. cell., 1·5–3·5 μ.

    Habitat.—On moist soil and rock, Naini Tal.

12. *Oscillatoria rubescens* D.C. Geitler, *op. cit.*,1930–32, p. 972, Figs. 620 *a-b*.

Lat. cell., 4·5–6·5 μ; long. cell., 1·5–3·5 μ.

Habitat.—On moist soil, Naini Tal.

13. *Oscillatoria variabilis* Rao. Rao, "The Myxophyceæ of the United Provinces, India—I," *Proc. Ind. Acad. Sci.*, 1936, **2 B**, p. 172, Fig. 3.

Lat. trich., 5·2–6·5 μ; long. cell., 3·4–5·0 μ.

Habitat.—On moist rock along with some desmids, Naini Tal.

14. *Oscillatoria chalybea* Mertens. Geitler, *op. cit.*, 1930–32, p. 956, Fig. 908 *b*.

Lat. trich., 7·5–8·5 μ; long. cell., 2·5–4·5 μ.

Habitat.—On stone in a waste-water drain, Naini Tal.

15. *Oscillatoria formosa* Bory. Geitler, *op. cit.*, 1930–32, p. 970, Fig. 619 *b*.

Lat. trich., 5·1–6·8 μ; long. cell., 1·7–3·4 μ.

Habitat.—On the pavement of a bathing ghat, Naini Tal.

16. *Oscillatoria quadripunctulata* Brühl and Biswas. Geitler, *op. cit.*, 1930–32, p. 966.

Lat. trich., 1·7–2·4 μ; long. cell., 3·4–5·1 μ.

Habitat.—On moist rock, Naini Tal.

17. *Phormidium jadinianum* Gom. Geitler, *op. cit.*, 1930–32, p. 1002, Fig. 640.

Lat. trich., 4·5–6·5 μ; long. cell., 1·5–3·0 μ.

Habitat.—On rotting logs along with moss plants, Naini Tal.

18. *Phormidium ambiguum* Gom. Geitler, *op. cit.*, 1930–32, p. 1015, Fig. 647 *e*.

Lat. trich., 3·4–5·1 μ; long. cell., 1·7–2·4 μ.

Habitat.—On moist soil, Naini Tal.

19. *Phormidium retzii* (Ag.) Gom. Geitler, *op. cit.*, 1930–32, p. 1012, Fig. 647 *a–d*.

Lat. trich., 4·5–10·5 μ; long. cell., 4·5–8·5 μ.

Habitat.—On moist rock, Naini Tal.

20. *Phormidium luridum* (Kütz.) Gom. Geitler, *op. cit.*, 1930–32, p. 1009, Fig. 645 *a*.

Lat. trich., 2·4–4·2 μ; long. cell., 2·4–3·4 μ.

Habitat.—On moist rock, Naini Tal.

21. *Lyngbya ceylanica* Wille. Geitler, *op. cit.*, 1930–32, p. 1054, Fig. 668 *a*.

Lat. fil., 15·5–24·5 μ; crass. vag., 3·5–4·5 μ; lat. trich., 9·5–15·5 μ; long. cell., 6·5–7·5 μ.

Habitat.—On moist soil along with other algæ, Naini Tnl.

22. *Lyngbya allorgei* Frémy. Geitler, *op. cit.*, 1930–32, p. 1059, Fig. 671.

Lat. fil., 6·5–7·5 μ; lat. trich., 4·5–6·5 μ; long. cell., 4·5–6·5 μ.

Habitat.—On moist rock along with *Marchantia* species, Naini Tal.

23. *Porphyrosiphon notarisii* (Menegh) Kütz. Geitler, *op. cit.*, 1930–32, Fig. 631.

Lat. fil., 18·5–19·5 μ; lat. cell., 8·9–14·5 μ; long. cell., 4·8–11·2 μ.

Habitat.—On moist soil, Naini Tal.

24. *Symploca muscorum* (Ag.) Gom. Geitler, *op. cit.*, 1930–32, p. 1122, Fig. 730.

Lat. fil., 5·1–5·9 μ; lat. trich., 4·2–5·1 μ; long. cell., 5·1–102 μ.

Habitat.—On moist soil along with moss plants, Naini Tal.

25. *Microcoleus sociatus* W. *et* G. S. West. Geitler, *op. cit.*, 1930–32, p. 1141, Fig. 746.

Diam. fil., 24·5–44·5 μ; lat. trich., 2·0–4·0 μ; long. cell., 4·5–8·5 μ.

Habitat.—On moist soil along with *Spirogyra* species, Naini Tal.

26. *Nostoc paludosum* Kütz. Geitler, *op. cit.*, 1930–32, p. 836, Fig. 528 *a*.

Lat. cell., 3·4–5·0 μ; long. cell., 3·5–6·5 μ; lat. het., 6·5–9·5 μ; long. het., 4·5–6·5 μ; long. spore., 4·5–12·5 μ.

Habitat.—On moist soil along with *Oscillatoria acuminata*, Naini Tal.

27. *Nostoc linckia* (Roth.) Born. *et* Flah. Geitler, *op. cit.*, 1930–32, p. 838, Fig. 528 *b*.

Lat. cell., 3·4–5·1 μ; long. cell., 4·2–5·9 μ; lat. het., 5·1–6·8 μ; long. het., 5·1–6·8 μ; lat. spore., 5·9–6·8 μ; long. spore., 6·8–8·5 μ.

Habitat.—On moist rock, Naini Tal.

28. *Nostoc commune* Vaüch. Geitler, *op. cit.*, 1930–32, p. 845, Fig. 536.

Lat. cell., 4·2–5·1 μ; long. cell., 4·2–5·1 μ; lat. het., 5·1–6·8 μ; long. het., 5·1–6·8 μ.

Habitat.—On damp soil, Naini Tal.

29. *Calothrix braunii* Born *et* Flah., Geitler, *op. cit.*, 1930–32, p. 606, Fig. 381.

Lat. fil., 9·5–10·5 μ; lat. trich., 6·7–7·5 μ; long. cell., 4·5–10·5 μ; lat. het., 6·5–8·0 μ; long. het., 4·5–7·5 μ.

Habitat.—On moist rock, Naini Tal.

30. *Scytonema mirabile* (Dillw.) Born. Geitler, *op. cit.*, 1930–32, p. 775, Fig. 498 *a-f.*

Lat. fil., 13·2–15·6 μ; crass. vag., 3·7–4·5 μ; lat. trich., 5·8–7·8 μ; long. cell., 2·5–3·5 μ; lat. het., 5·8–7·8 μ; long. het., 7·8–11·2 μ.

Habitat.—On moist soil along with other blue-green algæ, Naini Tal.

31. *Scytonema stuposum* (Kütz.) Born. Geitler, *op. cit.*, 1930–32, p. 756, Fig. 482 *a-b.*

Lat. fil., 19·8–23·5 μ; crass. vag., 1·9–4·8 μ; lat. trich., 12·5–17·8 μ; long. cell., 3·9–11·2 μ; lat. het., 12·5–17·5 μ; long. het., 11·2–17·8 μ.

Habitat.—On moist soil, Naini Tal.

32. *Scytonema ocellatum* Lyngbye. Geitler, *op. cit.*, 1930–32, p. 763, Fig. 488.

Lat. fil., 16·5–18·5 μ; crass. vag., 3·5 μ; lat. trich., 10·8–14·4 μ; long. cell., 7·5–10·8 μ; lat. het., 10·8–14·4 μ. long. het., 10·8–16·5 μ.

Habitat.—On moist soil along with moss plants, Naini-Tal.

33. *Scytonema hofmanni* Ag. Geitler, *op. cit.*, 1930–32, p. 772, Fig. 495.

Lat. fil., 9·5–10·5 μ; crass. vag., 1·5–1·7 μ, when old up to 2·5 μ; lat. trich., 6·5–7·5 μ; long. cell., 4·5–9·5 μ; lat. het., 4·5–5·0 μ; long het., 7·5–9·5 μ.

Habitat.—On moist rock, Naini Tal.

34. *Scytonema javanicum* (Kütz.) Born. Geitler, *op. cit.*, 1930–32, p. 765, Fig. 490.

Lat. fil., 15·5–18·5 μ; crass. vag., 3·5 μ; lat. trich., 9·5–10·5 μ; long. cell., 3·5–9·5 μ; lat. het., 9·0–9·5 μ; long. het., 9·5–10·5 μ.

Habitat.—On moist shaded rock, Naini Tal.

35. *Scytonema pascheri* Bhâradwâja. Bhâradwâja, "The taxonomy of *Scytonema* and *Tolypothrix*, including some new records and species from India and Ceylon," *Revue Algologique*, Paris, 1934, p. 158, Figs. *c–d*.

Lat. fil., 12·5–15·5 μ; lat. trich., 7·5–9·5 μ; crass. vag., 2·5–3·5 μ; long. cell., 4·5–9·5 μ; lat. het., 7·5–9·5 μ; long. het., 12·5–15·5 μ.

Habitat.—On moist soil, Naini Tal.

36. *Scytonema guyanense* (Mont.) Born. *et* Flah. Geitler, *op. cit*., 1930–32, p. 770, Fig. 493.

Lat. fil. 15·5–16·5 μ, when old up to 19·5 μ; crass. vag., 2·5–3·5 μ, when old up to 4·5 μ; lat. trich., 10·5–13·5 μ, when old narrowed down to 9·5 μ; long. cell., 4·5–15·5 μ; lat. het., 10·5–12·5 μ; long. het., 9·5–18·5 μ.

Habitat.—On moist soil, Naini Tal.

PRODUCTION OF GROWTH-PROMOTING SUBSTANCES BY FUSARIA AND THEIR ACTION ON ROOT ELONGATION IN *ORYZA SATIVA* L.

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Introduction

Interest has developed in recent years in the study of auxins and growth factors produced by fungi in culture, aimed at understanding the various physiological reactions induced in the host by fungal pathogens during disease incitation. Various reports cite instances wherein growth substances have been detected in fungus filtrates (Kalyanasundaram and Lakshminarayanan, 1953; Beckman, 1956; Fergus and Wharton, 1957). Certain antibiotics produced by fungi in culture have been found to produce growth response in wheat roots (Barton and MacNab, 1954). It has been suggested that synthesis of growth regulators by fungi during pathogenesis would alter host metabolism by stimulating the protoplasm of parenchyma cells, resulting in the formation of tyloses (Beckman, 1956). Studies on growth factor production by fungal parasites, both *in vitro* and *in vivo*, would therefore contribute to our knowledge on physiology of disease mechanism. The *in vitro* production of certain growth factors which stimulated rooting in cut shoots of host plants has been reported in several species of *Fusarium* (cited, Sadasivan and Subramanian, 1954). During an investigation on the influence of dialyzed culture filtrates of *Fusarium* spp. on root growth of *Oryza sativa* L. by a technique described earlier (Venkata Ram, 1956 *a*), it was observed that some of the fractions occurring in the filtrates, as for example certain amino acids, gave an increase in root growth corresponding to the cumulative effect produced by the culture filtrate, as already reported in brief by the author (Venkata Ram, 1956 *a*). Details of this work, as well as the results obtained in a study of the varietal interaction in *Oryza sativa* to response in root growth influenced by growth factors and certain amino acids, found to be present in *Fusarium* culture filtrates, are reported here.

Materials and Methods

Monoconidial cultures of Fusaria (obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland) were grown in 50 ml. Richards

medium in 250 ml. conical flasks for 3 weeks at 27–29° C., the fungal mat filtered and the filtrate dialyzed against distilled water. A 5% solution of the dialyzed filtrate (adjusted to pH 6·0) in distilled water (collected from an 'all-glass' Pyrex still) was tested for its action on root growth in rice plants, *Oryza sativa* L. The technique employed was the same as described earlier (Venkata Ram, 1956 *a*). Root growth was evaluated on the basis of mean root length of the seedlings obtained in each treatment and is given as percentage growth taking control, distilled water (untreated), as 100. The dialyzed filtrates were concentrated *in vacuo* and the various amino acids present in the filtrate determined by standard 2-dimensional chromatography (Block *et al.*, 1952) employing *n*-butanol: acetic acid: water (4: 1: 5) and phenol: water (3: 1) as solvents for the first and the second dimensions, respectively. The filtrate was also tested by standard bioassay techniques (György, 1950) for the presence of various B group vitamins employing *Phycomyces blakesleeanus* and standard mutants of *Neurospora crassa* and *N. sitophila*. All the vitamins and amino acids detected in the culture filtrate were similarly tested at various concentrations for their action on root growth. Richards mineral medium containing 50 g./l. of sucrose, glucose, fructose, arabinose and starch were compared for studying the effect of various C amendments in the media on the activity of their filtrates in influencing root elongation. Similarly, potassium nitrate in the mineral solution was replaced by ammonium nitrate, ammonium sulphate, urea and tryptophane, maintaining the concentration of nitrogen, potassium and sucrose at the level present in Richards medium, to give various N amended media. Urea was separately autoclaved and mixed with the sterilized medium under aseptic conditions. The initial pH of all the media was maintained at pH 5·0 and that of the filtrate adjusted to pH 6·0. Root tips taken from the seedling were cut to 5 mm. length and sectioned with a microtome. The cell length and width in the root tips up to 2 mm. and from 3–5 mm. from the tip were measured under a microscope.

The presence of growth-promoting substance in the fungus filtrate was also determined by the *Avena* cylinder technique (Thimann and Bonner, 1948). Selfed oat seeds were germinated over moist filter-paper exposed to red light for the first 48 hours. They were then allowed to grow in the dark in a humid chamber until the coleoptiles were 2–3 cm. long. The first 3 mm. of the tip was cut and discarded, the remainder of the coleoptile then microtomed into 3–4 sections of 3 mm. length. These were threaded on slender glass rods and floated in a shallow layer of the fungus filtrate in sterile Petri dishes. The strength of the filtrate was 10% in glass-distilled water and the pH 6·0. The entire operation was carried out under

red light and the elongaion resulting after 60 hours incubation measured under a microscope.

Results

The response in root growth of rice seedlings treated to 5% culture filtrate of Fusaria is shown in Table I. It was observed that out of the twenty-two species of *Fusarium* tested, filtrates of thirteen species increased root growth to more than 50% over the control. Culture filtrates of *F. udum* was inhibitory, whilst that of *F. equiseti*, *F. buharicum*, *F. conglutinans*, *F. bulbigenum* var. *lycopersici* and *F. orthoceras* caused only slight stimulation. Activity of the filtrates of *F. caucasicum*, *F. javanicum*, *F. vasinfectum*, *F. moniliforme*, *F. sambucinum* and *F. chlamydosporum* in increasing root growth was marked. In all these cases root growth was from 88–98% over the untreated seedlings, being almost twice that in the control. The taxonomic position of the *Fusarium* species did not seem to bear any relationship with the activity of their culture filtrates in influencing root elongation in rice seedlings.

The increase in root elongation in the seedlings treated to various culture filtrates was, however, not related to the dry weight of the root (Table I), as for example, in the case of *F. equiseti* and *F. buharicum* root weight was 63 mg. and 59 mg., respectively, and percentage growth was 110 and 109, whereas in *F. chlamydosporum* and *F. javanicum* the root dry weight was only 62 mg. and 60 mg., respectively, in comparison to a percentage growth of 188 in roots treated to their culture filtrate.

Four species of Fusaria, *F. vasinfectum*, *F. oxysporum*, *F. chlamydosporum* and *F. sambucinum*, were grown on Richards mineral medium containing various N and C sources and their culture filtrates tested for stimulation of root growth in rice seedling (variety CO17). Results are given in Table II. Synthesis of the growth factor(s) (referred to substances stimulating root elongation in the test plant) by the Fusaria varied markedly with the composition of the media. Incorporation of starch *in lieu* of sucrose in Richards medium resulted in maximum activity of the filtrate in the case of *F. oxysporum*, whereas it produced poorest growth response in the test plant when *F. vasinfectum* was employed. Maximum activity of *F. vasinfectum* filtrate was obtained in media containing arabinose as the carbon source, while glucose, fructose and sucrose also gave comparable results (Table II). The activity of the filtrate from cultures of *F. chlamydosporum* and *F. sambucinum* was high (170–220% growth) irrespective of the carbohydrate source employed.

TABLE I

Percentage growth of roots in 3-day old rice seedlings (variety CO17) treated to 5% culture filtrate of Fusaria and Avena *cylinders treated to 10% filtrate*

Fusarium spp.	Systematic group	Root growth in rice seedlings	Root dry weight (Total of 30 seedlings) mg.	Growth of *Avena* cylinders
F. solani	*Martiella*	179	58	113*
F. caucasicum ..	,,	190	69	..
F. javanicum ..	,,	188	60	112
F. orthoceras ..	*Elegans*	118	56	..
F. oxysporum ..	,,	140	67	101
F. conglutinans ..	,,	112	55	107
F. lini	,,	181	60	108
F. bulbigenum var. *lycopersici*	,,	115	53	103
F. udum	,,	78	46	107
F. vasinfectum ..	,,	188	67	109
F. moniliforme ..	*Liseola*	197	64	113
F. sambucinum ..	*Discolor*	198	69	109
F. culmorum ..	,,	172	61	..
F. buharicum ..	,,	109	59	..
F. dimerum ..	*Eupinnotes*	150	55	..
F. sporotrichioides ..	*Sporotrichiella*	179	66	..
F. chlamydosporum ..	,,	188	62	109
F. poæ	,,	128	57	..
F. semitectum ..	*Arthrosporiella*	162	69	..
F. equiseti	*Gibbosum*	110	63	..
F. scirpi	,,	168	66	..
F. lateritium ..	*Lateritium*	134	60	113

* Difference required for significance (P: 0·05) = 5; (P: 0·01) = 9.

TABLE II

Effect of culture filtrate, obtained from 3-week growth of Fusaria on various carbon and nitrogen amended Richards medium, on percentage root elongation in 3-day old rice seedlings (variety CO17) and growth of Avena *cylinder*

Nitrogen and Carbon amendments	Percentage root growth in rice seedlings				Percentage growth of *Avena* cylinders*	
	F. vasinfectum	*F. oxysporum*	*F. chlamydosporum*	*F. sambucinum*	*F. chlamydosporum*	*F. sambucinum*
KNO_3+sucrose ..	185	137	200	220	110	110
KNO_3+glucose ..	185	137	200	200	121	121
KNO_3+fructose ..	185	177	182	180	122	128
KNO_3+arabinose ..	200	151	180	170	..	..
KNO_3+starch ..	137	204	190	170	119	125
NH_4NO_3+sucrose	122	185	190	160	105	104
$(NH_4)_2SO_4$+sucrose	85	100	84	155	98	100
Tryptophane+sucrose	..	..	92	128	101	103
Asparagine+sucrose	155	100	182	175	119	119
Urea+sucrose ..	118	111	96	85	105	102

* Difference required for significance (P: 0·05) = 5; (P: 0·01) = 9.

F. oxysporum, *F. chlamydosporum* and *F. sambucinum* filtrates gave high activity when ammonium nitrate was substituted for potassium nitrate in Richards medium, whereas *F. vasinfectum* filtrate did not. Filtrate of only *F. sambucinum*, obtained from ammonium sulphate amended medium, stimulated root elongation, whilst filtrates of the other three Fusaria (Table II) were either inactive or toxic. With the exception of *F. oxysporum*, the other three organisms when grown on asparagine media gave filtrates which increased root elongation from 55–82% over the control. Tryptophane and urea, used as nitrogen sources, gave filtrates which had either poor or no activity, in all the four Fusaria.

The initial pH of the medium seemed to affect the activity of the filtrate in stimulating root elongation (Table III). Whereas, activity of *F. chlamydosporum* filtrate was consistently high, regardless of the initial pH of

the medium from pH 3·0 to 9·0, in the case of *F. moniliforme* there was a marked decrease in activity when the initial pH was increased from 6·0 to 7·0 and subsequently at the alkaline reactions (pH 8·0 and 9·0) the filtrate was toxic to root growth. A similar decline in the activity of the filtrate of *F. vasinfectum* was noticed when the initial pH was increased from 5·0 to 6·0 and from 6·0 to 7·0, but in this case activity of the filtrate increased thereafter at pH 8·0 and 9·0.

TABLE III

Relationship between various pH levels in Richards medium and the effect of their 3-week old culture filtrate on root growth of rice seedlings (variety CO17)

Initial pH	Percentage growth* in culture filtrates		
	F. vasinfectum	*F. chlamydosporum*	*F. moniliforme*
3·0	207	207	207
4·0	192	207	200
5·0	218	218	212
6·0	178	212	207
7·0	152	218	132
8·0	192	212	52
9·0	190	212	44

* Three-day old seedlings.

Results of chromatographic and microbiological assay of amino acids and vitamins present in 3-week old culture filtrate of *Fusarium* spp. are given in Table IV. The aim of this investigation was to analyse the vitamin and amino acid composition of *Fusarium* culture filtrate with a view to determine the effect of each of these constituents on root growth in *Oryza sativa* L. Since the activity of the *Fusarium* culture filtrate in stimulating root elongation was logically due to certain metabolites, either amino acids, vitamins, organic acids or other unknown factor(s), therefore, many of these substances, including all those detected in the filtrate (Table IV), were tried at various concentrations for activity in stimulating root growth in rice seedlings. Results are presented in Table V.

TABLE IV

Amino acids and vitamins detected by chromatography and bioassay present in 3-week old culture filtrate of Fusaria

Amino acids/ vitamins*	*F. lateritium*	*F. culmorum*	*F. vasinfectum*	*F. equiseti*	*F. javanicum*	*F. poæ*
Aspartic acid ..	×	×	×	×	×	×
Glutamic acid ..	×	×	×	×	×	×
Serine	×	×	×	—	×	×
Glycine	×	×	×	—	×	×
Threonine	×	×	×	×	×	×
Alanine	×	×	×	×	×	×
Lysine	×	×	×	×	×	×
Arginine	×	×	×	×	×	×
Proline	—	—	—	—	×	—
Valine ..	×	×	×	×	—	×
Leucine	—	×	×	—	×	×
Phenylalanine ..	×	×	×	×	×	×
Tyrosine	×	×	×	×	×	×
Cystine	×	×	×	—	×	—
Biotin	×	×	×	××	×	×
Choline	×	×	—	×××	×	××
Thiamine	×××	×××	×	×××	×××	××
Pyridoxine	×××	××	××××	××××	××××	××

* Relative concentration given.

None of the vitamins at the concentrations tried gave any marked increase in root growth of rice seedlings over the control. Thiamine at 60 p.p.m. appeared to be toxic. Sucrose and glucose at 0·25, 0·50 and 1·00% levels did not give much increase in root elongation. The Krebs cycle acids, succinic acid, fumaric acid, pyruvic acid and α-ketoglutaric acid, were ineffective in stimulating root growth at the concentrations employed in this experiment. Indole acetic acid and gibberellic acid inhibited root growth at concentrations higher than 1×10^{-7} and 1×10^{-8}, respectively, other strengths being ineffective. Potassium nitrate, magnesium sulphate

TABLE V

Percentage growth of roots in 3-day old rice seedlings (variety CO17) treated to inorganic nutrients, organic acids, amino acids, growth substances and vitamins

Substance	Concentration	Percentage growth	Substance	Concentration	Percentage growth
Biotin	10×10^{-6}	103	$FeCl_3$	1×10^{-6}	90
	10×10^{-7}	103		1×10^{-7}	105
	10×10^{-8}	111		1×10^{-8}	100
Thiamine	60×10^{-6}	70	Mn (as $MnSO_4$) ..	100×10^{-6}	98
	20×10^{-6}	100		50×10^{-6}	115
	10×10^{-6}	105		25×10^{-6}	105
	10×10^{-7}	110		10×10^{-6}	100
Pyridoxine ..	60×10^{-6}	118	Zn (as $ZnSO_4$) ..	100×10^{-6}	98
	20×10^{-6}	105		50×10^{-6}	110
	10×10^{-6}	100		25×10^{-6}	100
	10×10^{-7}	100		10×10^{-6}	100
Riboflavin ..	60×10^{-6}	98	B (as H_3BO_3) ..	100×10^{-6}	90
	20×10^{-6}	100		50×10^{-6}	98
	10×10^{-6}	105		25×10^{-6}	105
	10×10^{-7}	100		10×10^{-6}	100
Nicotinic acid ..	60×10^{-6}	100	Pyruvic acid ..	2×10^{-5}	100
	20×10^{-6}	105		1×10^{-5}	100
	10×10^{-6}	100		2×10^{-6}	100
	10×10^{-7}	100		1×10^{-6}	100
Choline	60×10^{-6}	105	Succinic acid ..	2×10^{-5}	100
	20×10^{-6}	100		1×10^{-5}	100
	10×10^{-6}	100		2×10^{-6}	100
	10×10^{-7}	100		1×10^{-6}	100
			Fumaric acid ..	2×10^{-5}	100
KNO_3	0·5%	120		1×10^{-5}	100
	0·25%	125		2×10^{-6}	100
	0·125%	105		1×10^{-6}	100
$MgSO_4$	0·125%	135	α-ketoglutaric acid	2×10^{-5}	100
	0·065%	115		1×10^{-5}	100
	0·035%	100		2×10^{-6}	100
				1×10^{-6}	100
KH_2PO_4	0·25%	115			
	0·125%	100	Indole acetic acid ..	1×10^{-6}	90
	0·065%	100		5×10^{-7}	98

TABLE V (*Contd.*)

Substance	Concentration	Percentage growth
Indole acetic acid ..	1×10^{-7}	100
	1×10^{-8}	100
	1×10^{-10}	110
	1×10^{-11}	100
Gibberellic acid ..	5×10^{-6}	90
	1×10^{-7}	98
	1×10^{-8}	105
	1×10^{-9}	100
Arginine	100×10^{-6}	62
	50×10^{-6}	78
	25×10^{-6}	78
	10×10^{-6}	100
Cystine	100×10^{-6}	126
	50×10^{-6}	150
	25×10^{-6}	133
	10×10^{-6}	110
	1×10^{-6}	100
Tryptophane ..	100×10^{-6}	88
	50×10^{-6}	110
	25×10^{-6}	100
	10×10^{-6}	100
Proline	100×10^{-6}	57
	50×10^{-6}	78
	25×10^{-6}	88
	10×10^{-6}	100
Aspartic acid ..	100×10^{-6}	57
	50×10^{-6}	62
	25×10^{-6}	88
	10×10^{-6}	100
Glycine	100×10^{-6}	119
	50×10^{-6}	125
	25×10^{-6}	110
	10×10^{-6}	100
Serine	100×10^{-6}	88
	50×10^{-6}	98
	25×10^{-6}	110
	10×10^{-6}	100
Lysine	100×10^{-6}	110
	50×10^{-6}	125
Lysine	25×10^{-6}	119
	10×10^{-6}	100
Threonine ..	100×10^{-6}	88
	50×10^{-6}	110
	25×10^{-6}	100
	10×10^{-6}	100
Valine	100×10^{-6}	88
	50×10^{-6}	105
	25×10^{-6}	100
	10×10^{-6}	100
Phenylalanine ..	200×10^{-6}	150
	100×10^{-6}	181
	50×10^{-6}	175
	25×10^{-6}	140
	10×10^{-6}	110
	5×10^{-6}	100
Alanine	100×10^{-6}	88
	50×10^{-6}	105
	25×10^{-6}	100
	10×10^{-6}	100
Glutamic acid ..	100×10^{-6}	40
	50×10^{-6}	57
	25×10^{-6}	87
	10×10^{-6}	100
nor-Leucine ..	100×10^{-6}	119
	50×10^{-6}	125
	25×10^{-6}	125
	10×10^{-6}	100
Tyrosine	200×10^{-6}	140
	100×10^{-6}	175
	50×10^{-6}	181
	25×10^{-6}	156
	10×10^{-6}	125
Sucrose	1·00%	115
	0·50%	108
	0·25%	100
Glucose	1·00%	105
	0·50%	115
	0·25%	100

and potassium phosphate (monobasic) were tested for activity at half, one-fourth and one-eighth the concentration, respectively, of that present in Richards medium. It was observed that magnesium sulphate at 0·125% and potassium nitrate at 0·25% gave an increase of 35 and 25%, respectively, in root growth, over the control (Table V).

The activity of certain amino acids in stimulating root elongation was found to be of considerable interest. Out of the fifteen amino acids investigated, at concentrations of 10, 25, 50 and 100 p.p.m., twelve gave little or no increase in root growth at any of the levels tried (Table V). The other three, namely, cystine, phenylalanine and tyrosine, markedly increased percentage growth in the seedlings treated to these amino acids. Cystine gave the maximum increase in growth at 50 p.p.m. level, being 50% over the control, whilst it was also active at 25 and 100 p.p.m. The response in root growth of rice seedlings treated to phenylalanine and tyrosine was very striking. Phenylalanine at 100 and 50 p.p.m. produced an increase of 81 and 75%, respectively, over the untreated control. Percentage increase in root elongation in seedlings treated to 100 and 50 p.p.m. tyrosine was 75 and 81% respectively. In both the amino acids maximum stimulation of root growth was achieved at concentrations of 100 and 50 p.p.m. although 25 p.p.m. also gave high activity.

In Table VI results are presented on the varietal interaction in *Oryza sativa* to stimulation of root growth by growth hormones, *Fusarium* culture filtrates, phenylalanine and tyrosine, the last two at concentrations which were found to be highly active on the variety CO17 (Table V). It was observed that out of the five varieties tested, only two, PTB10 and CO17, responded markedly to stimulation produced by *Fusarium* filtrates and the two amino acids (Table VI). Variety PTB10 also responded well to indole propionic acid but not IAA. Phenylalanine at 100 p.p.m. and tyrosine at 50 p.p.m., which increased root growth in varieties CO17 and PTB10 from 63 to 90% over the control, were found to be inhibitory to root elongation in the variety CH45 and caused no marked increase in growth in the varieties ADT2 and GEB24.

Linear and width measurement of cells in the root tips of untreated seedlings and those treated to *Fusarium* culture filtrate are given in Table VII. It was observed that in treated seedlings the cells were significantly longer than in the untreated control. As opposed to this, the width of the cells in the untreated roots was significantly greater than in those treated to the culture filtrate. The cells in the region 3–5 mm. away from the tip were significantly longer than those at the tip (0–2 mm.).

TABLE VI

Showing the response of different varieties of Oryza sativa *L. to stimulation of root growth by hormones, amino acids and culture filtrate of Fusaria*

Substance	Concentration	Percentage growth of roots in 3-day old seedlings				
		PTB10	CH45	ADT2	GEB24	CO17
Indole acetic acid	1×10^{-7}	85	89	110	100	100
	1×10^{-8}	85	84	120	122	100
	1×10^{-9}	85	84	100	115	100
Indole propionic acid	1×10^{-7}	163	100	100	100	100
	1×10^{-8}	143	122	120	122	100
	1×10^{-9}	111	112	100	122	100
Gibberellic acid	10×10^{-6}	1000	100	120	122	100
	25×10^{-6}	80	100	110	122	100
	50×10^{-6}	71	100	100	127	98
Phenylalanine ..	100×10^{-6}	165	92	98	122	190
Tyrosine ..	50×10^{-6}	163	88	100	127	185
Culture filtrate of *F. chlamydosporum*	5%	200	118	110	137	200
F. sambucinum	5%	188	111	115	118	195

DISCUSSION

The addition of 5 parts of dialyzed culture filtrate of many Fusaria to 95 parts of glass-distilled water markedly increased root growth of *Oryza sativa* L. (Table I). In some cases the response to stimulation by filtrate resulted in almost 100% increase in root elongation over the control in 3-day old seedlings (Tables I and II). However, root growth was not correlated with increase in dry weight (Table I). On the other hand, evidence obtained (Table VII) indicated that the increase in root length in the treated seedlings was primarily due to cell extension. Linear measurements of cells in the root tip gave significantly higher figures for roots responding to *Fusarium* culture filtrate as when compared to the control. Such a response to root growth as observed here simulates the action of the well-known auxins on plant cell. Previous workers have shown that the addition to mineral nutrient solution, of aqueous extract of leaf mould (Street, 1950), sterilized

TABLE VII

Length/width measurements of cells of root tips taken from 3-day old rice seedlings (variety CO17) responding to stimulation by 5% culture filtrate of F. chlamydosporum *in comparison to untreated seedlings*

	Mean* length of cell in microns		Mean width of cell in microns	
	Treated to culture filtrate	Untreated control	Treated to culture filtrate	Untreated control
0–2 mm. from the tip	29·70	26·40	9·30	19·00
	32·00	21·00	7·85	9·80
	38·10	14·90	9·45	11·00
3–5 mm. from the tip	50·30	30·50	6·85	12·60
	42·15	27·75	9·30	15·90
	77·70	24·10	10·05	13·00
	Difference required for significance (P: 0·05) = 11·5		Difference required for significance (P: 0·05) = 3·9	

* Each reading represents the mean of 30 measurements, 10 each from 3 different sections.

extract of horse dung (Chesters and Street, 1948) and aqueous extract of 'bacterized peat' (cited, Audus, 1953) markedly stimulated the growth of plants in the mineral solution. They attributed this action to certain organic growth substances occurring in the extract. Although mould and manure extracts are known to contain auxins and vitamins (Audus, 1953) it has been demonstrated that neither vitamin nor auxin were involved in the stimulation (Street, 1950). The hormone nature of the influence of *Fusarium* culture filtrate on root extension was not applicable in terms of an auxin response, since neither was any evidence obtained on the occurrence of indole compounds in the filtrate (unpublished data) nor was any response to indole acetic acid recorded (Tables V and VI). Street (1950) observed no significant stimulation of growth in lettuce and radish from applications of β-indolyl-acetic acid, α-naphthyl-acetic acid or naphthalene acetamide to the nutrient solution. Root curvature induced by growth substances, which were not related to indole acetic acid, have been shown to be present in culture filtrates of *Aspergillus niger* (Curtis, 1958).

Preformed vitamins of B group are known to enhance stem and root growth in plants (Schmidt, 1951; Audus, 1953) and are absorbed by the root system. However, vitamins of B group detected in *Fusarium* culture filtrate (Table IV), namely, biotin, thiamine, pyridoxine and choline, as also nicotinic acid and riboflavin, when tested individually, did not stimulate root growth markedly at any of the concentrations tried (Table V). The response in root extension when treated to the *Fusarium* filtrates could not, therefore, be ascribed to B vitamins occurring therein. Sucrose, glucose and the various organic acids investigated did not also seem to play any part in the stimulation of root growth (Table V).

Record of growth-promoting substance(s) occurring in yeast cells dates back to 1914 (cited, Audus, 1953). In recent years, Street (1950) observed that addition of yeast extract to nutrient solution produced a significant increase in the growth of radish and possibly certain of the amino acid constituents of the yeast extract produced the stimulation. In the present investigation amino acids occurring in the culture filtrate were tested individually at 10, 25, 50 and 100 p.p.m. concentrations for root growth response in rice seedlings (Table V). Striking response to cystine, phenylalanine and tyrosine treatments was achieved at 50 and 100 p.p.m. levels. Root extension in these three amino acid treatments was comparable to that achieved in the culture filtrates of certain *Fusaria* having high activity. An increase of 81% over the control in root elongation was noted in phenylalanine and tyrosine treatments. Stimulation of rooting in cut shoots of higher plants (cited, Sadasivan and Subramanian, 1954) and marked increase in respiration of host tissue (Lakshmanan and Venkata Ram, 1957) treated to *Fusarium* culture filtrates seems to be due to entirely different factors operative in the filtrate because such stimulation was not achieved with phenylalanine and tyrosine (Lakshmanan and Venkata Ram, 1957). The results reported in the present work seem to be the first record of stimulation of unexcised roots induced by amino acids, phenylalanine, tyrosine and cystine.

Data on straight growth of *Avena* cylinders treated to 10% filtrate of various *Fusaria* indicated that the factors inducing elongation in the *Avena* coleoptile sections differed from the ones stimulating root elongation in rice seedlings (Table I). Growth of *Avena* cylinders increased significantly in filtrates of *F. conglutinans* and *F. lateritium*, whereas root elongation in rice seedlings treated to these filtrates did not increase correspondingly. Filtrates of *F. udum* inhibited root elongation in rice seedlings, whereas it produced a significant increase in growth of *Avena* cylinders. Results obtained (Table II) also indicated that synthesis of growth substances to which a positive growth response in *Avena* sections was obtained, was

influenced by the composition of the growth substrate. Substitution of sucrose by either glucose, fructose or starch in Richards medium resulted in a significant increase in the activity of their filtrates to *Avena* coleoptile sections. Similar growth factors in fungus filtrates increasing growth of pea epicotyl sections have been previously reported in the case of *Ceratostomella ulmi* (Schwartz) Buisman (Beckman, 1956) and in *Ceratocystis fagacearum* (Bretz) Hunt (Fergus and Wharton, 1957). The fungal metabolites of *Puccinia carthami* have been noted to produce marked elongation in the infected host tissue (Daly and Sayre, 1957).

Root extension in response to N, P, K, Mg and Fe nutrition, supplied as inorganic salts, was either poor or not achieved (Table V). None of the trace elements at 10, 25, 50 and 100 p.p.m. exerted any marked stimulation. On the other hand, when the carbohydrate in the media was modified the filtrates varied in their activity. Culture filtrate of *F. vasinfectum* from starch substituted medium had lowest activity and that of *F. oxysporum* highest, in comparison to filtrates from either sucrose, glucose, fructose or arabinose amended media. Asparagine as the N source gave filtrates with high activity in the case of *F. chlamydosporum* and *F. sambucinum* and no activity in the filtrate of *F. oxysporum* (Table II). These observations indicated that certain organic substances in the filtrate, possibly phenylalanine, tyrosine and cystine, were responsible for stimulating root growth because the inorganic nutrient status of all the media was similar. The *in vitro* synthesis of amino acids by *Fusarium* mycelium is known to be influenced by the carbohydrate moiety in the media (Venkata Ram, 1956 *b*).

Host varietal response in *Oryza sativa* L. to *Fusarium* culture filtrates, phenylalanine and tyrosine, indicated that the root elongation achieved (Table V) mainly resulted from the interaction of the growth substances and the host cell because in three varieties of rice plants tested, little or no response to the filtrate and amino acids was observed (Table VI). The possibility of phenylalanine and tyrosine being converted to certain growth factor(s) through specific enzyme systems operating in the responding host variety suggested itself.

SUMMARY

The presence of growth-promoting substances in the culture filtrate of twenty-two species of Fusaria was detected by growth response produced in rice seedlings and *Avena* cylinders treated to the filtrate. A 5% solution of the dialyzed fungus filtrate produced marked elongation of the roots in 3-day old rice seedlings in the case of many *Fusarium* spp. Significant growth

response in *Avena* coleoptile sections was achieved in diluted filtrate of certain Fusaria.

Chromatographic and bioassay determinations revealed the occurrence of various amino acids and vitamins but not indole compounds in the filtrate. The action of these substances, as well as that of various organic acids, sugars, inorganic nutrients and growth hormones on root growth in rice seedlings was investigated. Little or no positive response in root elongation was achieved in all but three treatments, namely, cystine, phenylalanine and tyrosine. These three amino acids when employed at optimal concentrations induced an increase of 50–80% over the untreated control in root length, achieved in 3-day old rice seedlings. Such an increase in root growth corresponded to the cumulative effect produced by the *Fusarium* filtrate.

Stimulation of root growth induced by the fungus filtrate was not accompanied by a corresponding increase in accumulation of dry matter. On the other hand, most of the stimulation could be attributed to cell extension in the roots responding to the treatment. Evidence obtained indicated that certain organic constituents of the filtrate, which were synthesized by the fungus in the substratum, probably phenylalanine, tyrosine and cystine were responsible for causing the cell elongation which was also influenced by the composition and pH of the growth substratum.

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STUDIES ON THE OXYGEN CONSUMPTION IN TROPICAL POIKILOTHERMS

II. Oxygen Consumption in Relation to Body Size and Temperature in the Earthworm, *Megascolex mauritii*, when kept Submerged under Water

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Introduction

Although a large body of recent literature is available on the oxygen consumption in poikilotherms, comparatively little is known of this vital process in the lower terrestrial and amphibious organisms. It is of particular interest to study the respiration in amphibious forms like earthworms. Earlier investigations on the oxygen consumption of the worms have been confined mostly to the accessory respiratory organs, especially in tubicolous worms (Alsterberg, 1931; Zoond, 1931). Almost all of the recent investigations have dealt with oxygen consumption in the earthworm in relation to oxygen tension and habitat (Dolk and Paauw, 1929; Johnson, 1942; Koenen, 1951; Beadle, 1957).

Ralph (1957) studied the oxygen consumption of the earthworm, *Lumbricus terrestris*, in relation to its activity. He showed that the diurnal rhythms of activity and oxygen consumption were related to the lunar cycle.

Krüger (1952), in a careful study, demonstrated that the oxygen uptake varies with body surface in the earthworm, *Eisenia fætida*, at 15° C. In a recent study of the influence of temperature on metabolism Kirberger (1953) has presented data on the oxygen consumption in relation to temperature, in *Lumbriculus variegatus* and *Eisenia fætida*. But Kirberger's data are not weight-controlled while Krüger's studies are confined only to a single temperature.

The brief review given above shows that oxygen consumption in the earthworms has not been closely investigated in relation to body size and temperature. In this paper I have tried to elucidate the effect of size as well as temperature on oxygen consumption in *Megascolex mauritii* when submerged under water.

MATERIAL AND METHODS

Megascolex mauritii, very common under moist soil in this locality, were collected at intervals from about the same spot to avoid any possible variations due to difference in habitat and nutrition.* They were kept in glass troughs in the laboratory, with blotting-paper strips, completely immersed in tap water, which was changed once daily in the mornings. No linear or other measurements of the worms were taken for 2 or 3 days after collection till all the muddy contents of the gut were replaced by the blotting-paper pulp. The worms continued to live in the laboratory under the above conditions without food for several days.

All the measurements were made during September and October, when the room temperature remained around $28 \pm 1°$ C. Worms weighing from 0·15 to 1·5 gm. were used in the experiments.

The respiratory chambers consisted of wide mouthed bottles of 150 and 200 ml. capacity. The size of the bottle was such that it was not too big for the enclosed worm (which was generally inactive) to give a considerable difference in the oxygen content. The chamber (3) (*see* Fig. 1) was closed with a rubber stopper which was provided with an inlet (2) and

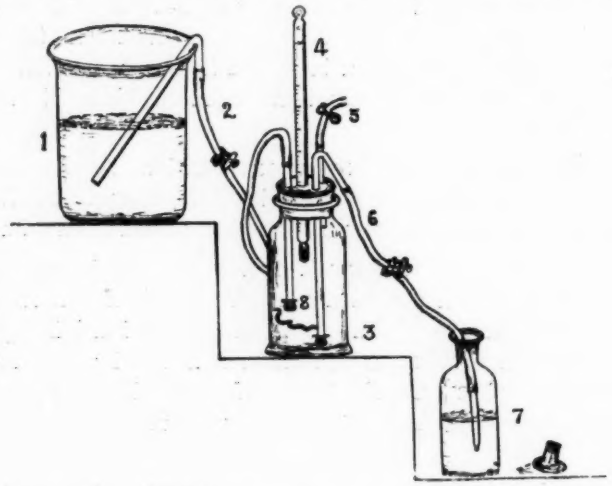


FIG. 1. Diagram showing the arrangement of the respiratory chamber. For explanation please see text.

* I am indebted to the Director, Zoological Survey of India, Calcutta, for the identification of the earthworms.

an outlet (6). The open ends of these inlet and outlet tubes within the respiratory chamber were covered with wire-gauze (8) to prevent the earthworm from entering these tubes. It was also provided with a small tube (5) to find whether the chamber was water-tight or not and a thermometer (4) to show the temperature of the water in the chamber. The inlet was connected to the reservoir (1), and through the outlet the sample was drawn into glass-stoppered bottles (7). After the chamber was found water-tight with the worm inside, the initial sample was drawn into the sample bottle the end of the outlet always keeping below the water level in the sample bottle. The outlet was also closed by a screw cock and then the inlet too was closed. At the time of drawing the final sample after an hour's interval the small tube was opened by removing the pinch cock in order to let water out through the outlet tube which was also open. The water in the respiratory chamber was thus drawn out into the sample bottle. The general set-up of the respiratory chamber and its connections are shown in Fig. 1.

TABLE I

Regression coefficient of oxygen consumption in relation to weight (b-*value*) *at different temperatures*

Temperature ° C.	15	20	25	30	35
b-Value ..	0·874	0·745	0·628	0·623	0·560

Only one individual was put in each respiratory chamber and the duration of each experiment was one hour. The whole assembly was held in the refrigerator or the hot both as the case may be and maintained at the required temperature constant to $\pm$ 1° C. For each individual the oxygen consumption was acutely measured at five temperatures from 15° C. upward as follows: 15, 20, 25, 30 and 35° C. To avoid activity due to light the chambers were coated with black paint and the animal left in the running water for 10 or 15 minutes after it was placed in the water-tight chamber to become adapted to the black chamber. Winkler's method as given in Welsh and Smith (1953) was employed for measuring dissolved oxygen. The oxygen consumption at each temperature was calculated from the difference in oxygen content between the initial and the final samples.

RESULTS

Oxygen consumption in relation to size.—The data are summarized in Fig. 2 and in Table II. It may be seen from the figure that at all tempera-

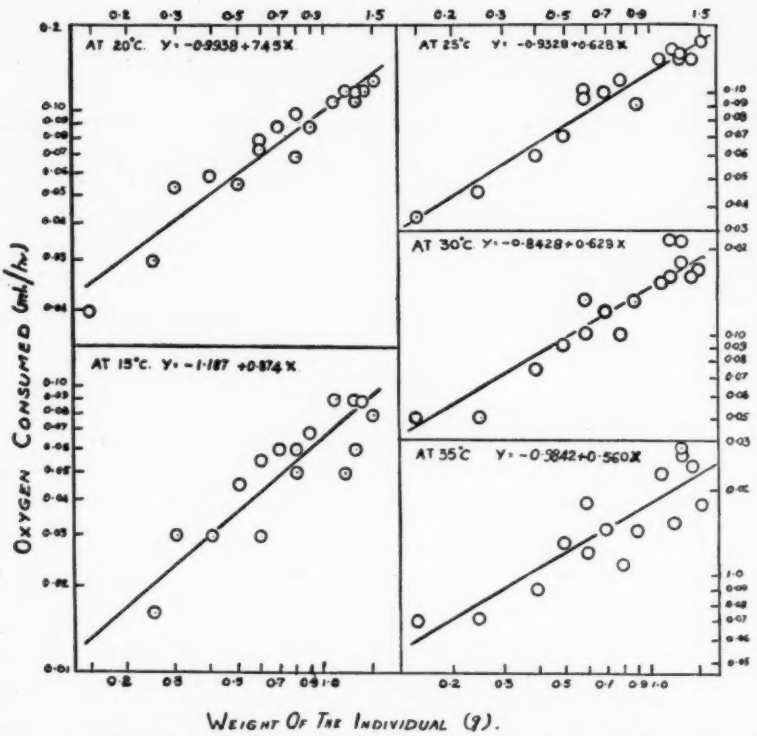

AT 20°C. Y = -0·9938 + 7·45X
AT 25°C Y = -0·9328 + 0·628X
AT 30°C Y = -0·8428 + 0·629X
AT 15°C. Y = -1·187 + 0·874X
AT 35°C Y = -0·3842 + 0·560X
OXYGEN CONSUMED (mL/hr.)
WEIGHT OF THE INDIVIDUAL (g).

FIG. 2. Oxygen consumption as a function of body weight, at different temperatures, in the earthworm *Megascolex mauritii*. Double logarithmic grid. Please note that the scales on the Y-axis start at different levels in the different cases.

tures oxygen consumption increases with increasing weight of the animal. But the rate of this increase or in other words the regression of oxygen consumption on weight is not the same at all temperatures. The *b*-values listed in Table I show that the highest value is obtained at 15° C. and it consistently decreases with increasing temperature (Fig. 4).

As is to be expected from the above-given regression values, the oxygen consumption per unit weight decreases with increasing weight of the individuals (Table III), and the rate of this decrease is highest at the extreme high temperature.

At lower temperatures (*vide* Table III) (15 to 20° C.) there occurs nearly a 50 per cent. fall in the rate of oxygen consumption of smaller worms (below 0·5). But the decrease in the rate of oxygen consumption is not so great

TABLE II

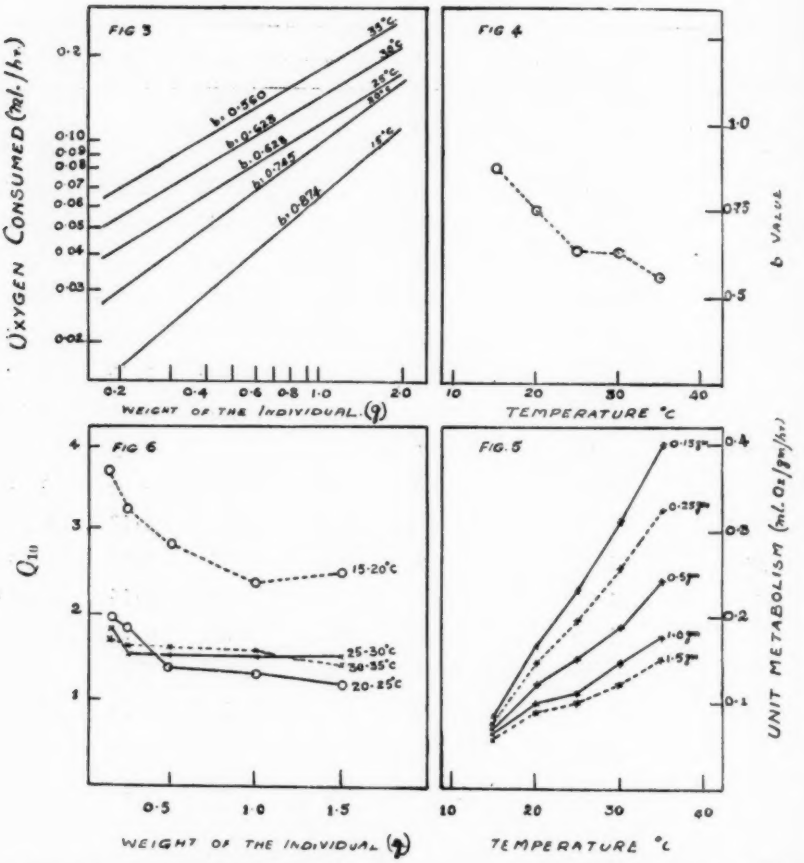
Oxygen consumption of the earthworm, Megascolex mauritii, *in relation to different sizes and different temperatures*

Sl. No.	Wt. of the earthworm in gm.	Oxygen consumption (ml./hr.)				
		15° C.	20° C.	25° C.	30° C.	35° C.
1	0·15	0·01	0·02	0·036	0·05	0·07
2	0·25	0·016	0·03	0·045	0·05	0·07
3	0·30	0·03	0·055	..	..	..
4	0·40	0·03	0·06	0·06	0·076	0·09
5	0·50	0·046	0·056	0·07	0·09	0·13
6	0·60	0·055	0·08	0·096	0·10	0·12
7	0·70	0·06	0·09	0·10	0·12	0·147
8	0·80	0·06	0·10	..	..	..
9	0·80	0·05	0·07	0·115	0·10	0·11
10	0·90	0·07	0·09	0·09	0·13	0·145
11	1·10	0·09	0·11	0·13	0·15	0·23
12	1·20	0·06	0·12	0·14	0·16	0·155
13	1·30	0·09	0·12	0·13	0·18	0·28
14	1·30	0·06	0·11	0·135	0·21	0·265
15	1·40	0·09	0·12	0·13	0·16	0·24
16	1·50	0·08	0·13	0·15	0·17	0·18
17	0·60	0·03	0·076	0·101	0·13	0·18

in the larger individuals. Further, the larger individuals do not show a conspicuous increase in oxygen consumption from 20 to 25° C.

Oxygen uptake in relation to temperature.—All the individuals studied, excepting one (1·2 gm. animal at 35° C., *vide* Table II) show an increase in oxygen consumption with increasing temperature over the range of temperatures studied. It is evident from Fig. 5, that the R-T curves for individuals of different sizes do not look similar but individuals of all sizes exhibit a relatively uniform decline in their rate of oxygen consumption over almost the whole range of temperatures studied, excepting from 20 to 15° C., in

which range they exhibit a sharp drop in oxygen consumption below 20° C. (*vide* Table III).



FIGS. 3–6. Fig. 3. Comparison of oxygen consumption of the earthworm at different temperatures. Curves taken from Fig. 1. Fig. 4. *b*-Value (Regression coefficient of oxygen consumption in relation to weight) as a function of temperature. Fig. 5. Rate of oxygen consumption as a function of size and temperature. Fig. 6. Q_{10} of oxygen consumption in relation to size and temperature.

As will be seen from Fig. 4 and Table I the *b*-value, in this case, decreases as the temperature of the medium increases.

These tendencies are more clearly brought about if Q_{10} is considered (*vide* Table IV) as shown below,

TABLE III

Rate of oxygen consumption in relation to size and temperature

Sl. No.	Wt. of the earthworm in gm.	Unit metabolism (O_2 ml./gm./hr.)				
		15° C.	20° C.	25° C.	30° C.	35° C.
1	0·15	0·0867	0·1667	0·2333	0·3133	0·400
2	0·25	0·0785	0·1446	0·1956	0·2520	0·3285
3	0·50	0·0719	0·1212	0·1511	0·1865	0·2422
4	1·00	0·0659	0·1016	0·1168	0·1436	0·1785
5	1·50	0·0580	0·0916	0·1003	0·1232	0·1494

TABLE IV

Q_{10} of oxygen consumption in relation to size and temperature

Sl. No.	Wt. of the earthworm in gm.	Q_{10}			
		15–20° C.	20–25° C.	25–30° C.	30–35° C.
1	0·15	3·648	1·960	1·802	1·708
2	0·25	3·406	1·828	1·540	1·638
3	0·50	2·839	1·357	1·524	1·611
4	1·00	2·375	1·323	1·511	1·545
5	1·50	2·496	1·199	1·508	1·471

Q_{10} of oxygen consumption in relation to temperature and size.—The Q_{10} values have been worked out only for 5 different sizes and are summarized in Table IV and Fig. 6. The Q_{10} decreased with the increase in size at all temperatures studied. As is to be seen from the Q_{10} values given, there is no systematic change in Q_{10} with increasing temperature.

For all sizes, the Q_{10} is exceptionally greater at the extremely low temperatures between 15 and 20° C. Further it may be seen that the smaller individuals below 0·5 gm. exhibit the highest Q_{10} which is round about 3·5,

whereas the Q_{10} of the larger worms (above 0·5 gm.) within the same temperature range is round about 2·5. These higher Q_{10} values are also evident from the low rates of oxygen consumption at 15° C.

DISCUSSION

The oxygen uptake in relation to size has been much investigated. Many authors have referred to the surface relationship of oxygen consumption. It is known that the regression gradient or *b*-value is not a constant value for the same species under different environmental conditions (Rao and Bullock, 1954; Rao, 1958) and at different developmental stages, reproductive phases, etc. (Zeuthen, 1955). In this case, as is shown in Fig. 4, temperature has a direct effect on the slope of the regression lines. The *b*-values at 25 and 30° C. (0·628 and 0·623) indicate that the oxygen uptake is proportional to the surface area of the worm.

It can be said from the *b*-values, that the oxygen consumption is proportional to the surface area in temperatures round about the habitat temperature, *i.e.*, 25 and 30° C. The aquarium temperature in which the worms were kept was 28 ± 1° C. This agrees with the results reported for *Eisenia fœtida* by Krüger (1952). But it is noteworthy that the *b* increases as the temperature of the medium is decreased below the habitat temperature and *vice versa*. Considering the facts mentioned above, it may be said that the weight regression of oxygen consumption is greatly dependent on the temperature at which such relationships are measured.

Worms have shown high Q_{10} values at the lower temperature between 15 and 20° C. acutely measured and also Q_{10} decreases at all temperatures with increasing size (Table IV). Alsterberg (1922) working on Tubificidæ and Konopacki (1907) studying the physiology of respiration in Lumbricidæ showed that the frequency of the rhythm of oscillation in Tubificidæ varies at different temperatures with $Q_{10} = 2·1$ and the rate of respiration in the Lumbricidæ varies directly with temperature, having a $Q_{10} = 2$ within the limits of 2·5 to 29·5° C. Kirberger (1953) also reported similar values of Q_{10} in *Eisenia fœtida* and *Lumbriculus variegatus*. But all the above values for Q_{10} are not size-controlled. In the present case Q_{10} varies irregularly with a rise in temperature (*vide* Table IV) and is normally less than 2 excepting below 20° C. where cold depression seems to set in.

From the data of Edwards and Irving (1943), Rao and Bullock (1954) showed that in *Talorchestia* Q_{10} increased with size between 12 and 22° C. in summer individuals. In winter animals between 7 and 17° C., Q_{10} increases with size, but at 23 and 32° C. the very steep regression of unit metabolism

indicates the heat depression of larger individuals. In the present case, too, there occurs a steep decline in the regression gradient at 35° C. which may be due to heat depression of larger worms. This is further supported by the wider scattering of the points in the larger sizes at 35° C.

The increase in the regression gradient at 20 and 15° C. may be explained by the higher Q_{10} values. It will be seen from Table III, that there is a decrease in metabolic rate of the smaller worms (below 0·5 gm.) from 25 to 20° C. In the larger worms the decline is not significant. This is also clearly expressed by the higher Q_{10} values between 25 and 20° C. (*vide* Table IV) of smaller worms. By this, the upper end of the curve remains nearly the same at 20° C. as it has been at 25° C. (*vide* Fig. 3), whereas the lower end of the curve is pulled down due to the decrease in the metabolic rate of smaller worms. This results in a curve with a higher *b*-value as seen in Fig. 3. The same trends continue to manifest at 15° C. in a much more conspicuous manner, as could be seen from the Q_{10} values for 15 to 20° C. Here the decline in metabolic rate is steep in all sizes, but distinctly greater in smaller worms as shown in Tables III and IV, the Q_{10} for smaller worms being more than 3, while the larger worms have a Q_{10} around 2·5. Hence the lower end of the regression line is depressed much more than the higher end. Therefore the curve is more steep at 15° C. than at 20° C., with a higher *b*-value (0·891).

The decrease in *b*-value at 35° C. appears to be mainly due to the greater decrease in metabolic rate of the larger worms above 1·00 gm. at this temperature as shown in Table III. As has been mentioned earlier, this may be due to the heat depression of larger worms at 35° C.

General conclusions may be drawn on the effects of habitat temperature on Q_{10} and regression values, only when populations of worms are studied during the different seasons (summer and winter).

Summary

The oxygen consumption of *Megascolex mauritii* was measured at 15, 20, 25, 30 and 35° C. during summer with worms varying in weight from 0·15 to 1·5 gm. They were kept immersed under water at the laboratory temperature (at 28 ± 1° C.).

The size-temperature relationship for oxygen consumption revealed a proportionate rise in oxygen consumption at higher temperatures. But the relative rate of increase is greatest in smaller worms.

Q_{10} is discussed in relation to size and sudden changes in temperature and it was shown that it increases with decreasing weight. No systematic variation in Q_{10} with temperature was noticed.

The regression coefficient for oxygen consumption in relation to weight shows a consistent decrease with increasing temperature over the range 15 to 35° C. But at 25 and 30° C. which approximate the habitat temperature, the oxygen consumption of *Megascolex mauritii* shows surface proportionality.

The changes in the *b*-values (regression coefficient) are explained on the basis of observed Q_{10} values.

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‡ The papers by Alsterberg (1922) and Konopacki (1907) cited in this reference have not been consulted in the original.

CYTO-GENETIC STUDIES IN INDIAN HONEY-BEES

I. Somatic Chromosome Complement in *Apis indica* and its Bearing on Evolution and Phylogeny

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I. Introduction

The genus *Apis* L. comprises four well defined species *Apis florea*, *A. dorsata*, *A. indica* and *A. mellifera*. Systematic classification of subspecies, varieties and races of the last two species is rather ambiguous and this has been critically reviewed by Ruttner and Mackensen (1952) as also Kerr and Laidlaw (1956). *A. florea* and *A. dorsata*, which are the most primitive members of the genus, are mostly confined to Indo-Malayan regions. *A. indica* is distributed over a wide range in South-East Asia including India, Ceylon, Malaya, Burma, China, Java, Philippines and even Japan. In India and adjacent Pacific islands, *A. indica* occurs in three distinct forms: the darker *picea* a variety common along higher altitudes, the lighter *indica* mostly confined to lower plains and the intermediate *peroni* distributed along the transitions. Intensely hairy forms of *A. indica* occurring in major parts of China and Pacific Islands like Java, Philippines and Japan have been classed as varieties *sinensis*, *javanica*, *phillipina* and *japonica* respectively. The last one has been raised to specific rank of *A. japonica* Radosz by Tokuda (1935) on the basis of bionomic differences and non-crossability with European bees.

Primary distribution of *A. mellifera* covers Central Asia and Mediterranean regions from where its migration has extended over the continents of Africa and Europe. In Africa, it has differentiated into varieties: (1) *fasciata*, (2) *intermissa*, (3) *adensonii*, (4) *unicolar* and (5) *capra*. In Central Asia and Europe, it has differentiated into: (1) *caucasica*, (2) *syriaca*, (3) *ligustica* (Italian), (4) *carnica*, (5) *mellifica*, (6) *lehzeni* (Scandinavian), (7) *silvarum* (Russian) and (8) *acervorum* (Balkan) (Ruttner and Mackensen, 1952).

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Distinction between *A. indica* and *A. mellifera* has mostly quantitative rather than qualitative basis and forms of *A. indica* along the North-West Frontiers in the Himalayas closely resemble *A. mellifera*. Hatch (1950) actually mentions that *A. mellifera* occurs in India. The two species freely hybridize and workers of one care for the queens of the other (Muttoo, 1951). For these reasons, these two have been often merged into a single species (Kerr and Laidlaw, 1956).

Alpatov (1925) observed correlation between geographical distribution and body-size of *A. mellifera* which increases with northern latitudes in Russia. Michailoff (1928) even gave a factor which permitted calculation of body-size along the geographical latitudes in Russia. Since distinction between *A. indica* and *A. mellifera* has mainly quantitative basis, Alpatov's generalization may as well be extended further south to *A. indica* in India, where also there is a progressive increase in body-size along northern latitudes and higher altitudes. In fact, the entire distribution of *A. indica* and *A. mellifera* can be considered as a continuum of a single species which has differentiated into subspecies, varieties and races during the course of its migration through the three main migratory routes along (1) China, Japan and Pacific Islands, (2) Egypt and Africa and (3) Central Asia and Europe.

Since the natural distribution of the genus *Apis* shows maximum diversity in India and adjacent areas, these regions constitute the centre of origin and early evolution of this genus.

II. Previous Cytological Work

The earliest cytological observations on *Apis mellifera* have been reported by Petunkewitsch in 1901–03 followed by Doncaster, Marl and Coopland, Meves, Granata, Nachtsheim, Jegen and Sanderson in later years. Sanderson and Hall (1948) as also Whiting (1945) have reviewed the work of all these earlier workers and have presented observations on somatic and gametic chromosomal constitution in *A. mellifera*. These authors have given critical evidence for occurrence of 16 chromosomes in the haploid males and 32 in female as normal numbers in blastoderm and gonical cells. Abnormal polysomatic numbers have been observed in hypodermal, malphigian, trachial and follicular epithelial cells of testes and ovaries. The mitotic chromosomes are thinner, longer with distinct difference in size, shape and individuality.

Sanderson and Hall (1952) have studied chromosome constitution of *A. mellifera unicolor* variety *intermissa* from South Africa. This species has also haploid males with 16 and diploid females with 32 somatic chromo-

somes. Hashinohe and Onishi (1952) have reported 16 and 32 as somatic numbers for haploid males and diploid females for the Japanese honey-bee and have studied chromosome behaviour during spermatogenesis. Existence of a differential sex chromosome in the honey-bee first observed by Manning (1950) and confirmed by Kerr (1951) has been subsequently refuted by Kerr and Hans (1952), where they state that the so-called 'X' chromosomes may only be a persistent nucleolus, being feulgen negative. There is considerable genetic evidence to show that sex in honey-bee is neither dependent on sex chromosomes nor on gene balance, but that is controlled by a series of multiple sex alleles as in *Habrobrachon* such that all heterozygotes are females and homo as well as hemizygotes are males (Whiting, 1943; Mackensen, 1951). On the basis of pairing in haploid males first observed by Nachtsheim (1913), Whiting (1945) and Kerr (1952) infer polyploid nature of *A. mellifera*.

Some species from the closely related genera *Osmia*, *xylocopa* and *euplasia* also show a somatic complement of 32 in female and 16 in haploid male (Armbruster, 1913; Granata, 1909 and Makino, 1951). Five species of *melipona* as also *Trigona mosquito* studied by Kerr (1948) show a somatic complement of 18 in females and 9 in haploid males in all cases. *Bombus fervidus* has 12 chromosomes in the somatic complement of both males and females (Kerr, 1948).

No cyto-genetic work has been so far reported on the three Indian species of honey-bee *A. indica*, *A. florea* and *A. dorsata*. These have been taken up for investigation in this laboratory along with some Indian members of closely related genera like *Trigona*. Observations on somatic chromosome complement in *A. indica* are presented here.

III. MATERIAL AND METHOD

Worker and drone-eggs of *A. indica* as also their larvæ at various developmental stages were pretreated in Carnoy's fluid for a few seconds and then fixed in Navashin's fluid for 24 hours. The fixed material was then washed in several changes of water, dehydrated in graded acetone, passed through grades of chloroform and embedded in paraffin. Sections were cut 12·5 microns thick and stained with crystal violet or hæmatoxylin.

Camera lucida drawings were made at bench level using fluotar oil immersion 100× and a compensating eye-piece 25× giving a final magnification of 3,700 approximately. Photomicrographs were taken with the same oil immersion combined with photo-eye-piece 10× and then enlarged to magnifications indicated in the explanatory note to the plate.

IV. Somatic Chromosome Complement in the Female *A. indica*

During the earliest stages of embryonic development in the worker eggs, about 24 to 72 hours old, the outermost epithelial layer of the blastoderm shows clear mitotic plates, each showing 32 chromosomes distinctly (Fig. 1, Photographs 1 *a* and *b*). The chromosomes are very slender and small with an average breadth of about 0·25 microns. Lengths of individual chromosome ranges from 0·75 to 1·5 microns only. The chromosomes have distinct morphological individuality and they show somatic association of two, three or four homomorphic members. Out of the somatic complement of 32 chromosomes, there are about 8 chromosomes with median, 8 with sub-median and 16 with sub-terminal constrictions. Due to very short lengths, chromosomes with sub-terminal and sub-median constrictions are often confused for each other.

V. Somatic Chromosome Complement in Male *Apis indica*

In the drone-eggs, during first 24 to 72 hours, epithelial layers of the blastoderm show clear mitotic plates each showing only 16 chromosomes. Average breadth of individual chromosome is about 0·25 microns and their length ranges from 0·75 to 1·5 microns. Somatic pairing is more conspicuous in the male and the 16 chromosomes show distinct somatic association in 8 homomorphic pairs (Fig. 2, Photographs 2 *a*, *b* and *c*). Morphological analysis and relative dimensions have been schematically indicated for the somatic complement in the haploid male (Fig. 3). Relative measurements of the two arms and total lengths of individual chromosomes have been tabulated in Table I.

Somatic complement in the haploid male thus consists of two pairs with median, two with sub-median and four with sub-terminal constrictions (2 *m.c.* + 2 *s.m.c.* + 4 *s.t.c.*).

VI. Polysomaty

The normal somatic chromosomal counts of 16 in male and 32 in female are observed mostly in the blastoderm stages and that too confined to the outermost epithelial layers. With further embryonic developments, excepting the germinal layers of testes and ovaries, most other tissues show progressive polysomaty and the individual cells contain chromosomes in multiples of the basic 16 number. Such a polysomaty is usually of euploid type, particularly in earlier stages of repeated duplications. During such multiplications, the nuclear cavity enlarges but the nuclear membrane remains intact and the chromosome number increases through repeated endomitosis. The individual chromosomes are highly condensed and most often reveal

TABLE I

Karyo-type analysis in the haploid male A. indica

Pair No.	Position of construction	Length of two arms, microns	Total length microns
I	Median	0·75+0·75	1·50
II	Sub-median	1·00+0·50	1·50
III	Sub-terminal	1·00+0·25	1·25
IV	Sub-terminal	1·00+0·25	1·25
V	Sub-terminal	1·00+0·25	1·25
VI	Sub-terminal	0·75+0·25	1·00
VII	Median	0·50+0·50	1·00
VIII	Sub-median	0·50+0·25	0·75

Total length at m-phase.....$9 \cdot 50 \times 2 = 19$ microns.

a distinct longitudinal split. Besides the nuclear membrane, the individual chromosomes or a group of homologues are enclosed in separate vesicles especially during earlier stages. There is synchronization of successive divisions in each vesicle so that they contain about the same number of chromosomes in each. No such synchronization is seen at later stages so that different vesicles inside a nuclear cavity may contain different numbers thus leading to aneuploid numbers which may not be multiples of the haploid set. With progressive augmentation of chromosome numbers, separate vesicles enclosing the homologous groups fuse together and highly condensed chromosomes in large and indefinite numbers form random clusters making the counts impossible. Development of polysomaty in different tissues during embryonic growth is being critically studied and detailed observations will be presented later.

VII. AFFINITIES BETWEEN *A. indica* AND *A. mellifera*

Present observations on somatic chromosome complement in *A. indica* show that it closely resembles *A. mellifera* in respect of: (1) chromosome number with 16 in male and 32 in female, (2) haploidy in male and diploidy in female, (3) high prevalence of polysomaty in most of the tissues excepting

the blastoderm epithelium and gonial tissues. This, therefore, confirms the close taxonomic affinities between *A. indica* and *A. mellifera* as inferred by earlier workers on the basis of comparative anatomy and behaviour pattern.

Morphological analysis of the karyo-type of *A. indica* has been attempted here for the first time. As similar data on karyo-type analysis in *A. mellifera* are not available so far, it is not possible to compare the morphology of individual chromosomes in these two species. Such a comparison along with meiotic chromosome behaviour in their hybrids would indicate the extent of structural differentiation in the karyo-types of *A. indica* and *A. mellifera.*

VIII. Polyploid Constitution of Domesticated Bees

Reference to the idiogram of the haploid male complement of *A. indica* reveals a very interesting and significant situation. In spite of the fact that the normal somatic complement in the male is haploid relative to the normal number in the female, the 16 chromosomes in the haploid set clearly occur as 8 homomorphic pairs. If such homomorphic condition implies gross genetic homology, it may be inferred that the male is numerically haploid but genetically diploid, the female being a tetraploid.

Whiting (1945) has inferred from the earlier chromosome drawings of Nachtsheim (1913) that the male and female in *A. mellifera* are diploid and tetraploid respectively with 8 as the basic number. This inference is based on pairing in haploid during spermatogenesis. But Sanderson and Hall (1948) do not agree with Whiting's inference as they did not observe any pairing in the male to suggest its diploid constitution. Karyo-type analysis of the somatic complement in male *A. indica* attempted here suggests the possibility of male and female being genetically diploid and tetraploid respectively. Whiting's inference is, therefore, in agreement with the observations presented here.

Genetic studies on *A. mellifera* also suggest its polyploid constitution. Critical reviews by Ruttner and Mackensen (1952) and Kerr and Laidlaw (1956) reveal that many of the characters in *A. mellifera* are controlled by multiple genes without linkage. In contrast to this, most of the characters in related genera like *Melipona* or *Trigona* with lower chromosome numbers, show simple mono or dihybrid segregation. Thus, for instance, in *A. mellifera* at least seven gene loci exist for yellow abdomen colour (Roberts and Mackensen, 1951), several genes control tongue length (Michailoff, 1928) and four eye colour mutant genes ivory (*i*), snow (*s*), cream (*cr*), and yellow-green (*ch*), are all recessive to normal and non-allelic without any linkage between them four, the first three being epistatic to the last (Rothenbuhler *et al.*, 1952).

A further confirmation of such a polyploid constitution is indicated from the cyto-genetic studies on *A. florea* in progress at this laboratory. Tentatively, it seems that the small Indian bee *A. florea* has haploid males and diploid females with 8 and 16 chromosomes respectively, as their normal somatic numbers, apart from high incidence of polysomaty as in other *Apis* species. These observations are being critically pursued further to avoid any confusion due to polysomaty.

Lindauer (1957) also considers *A. florea* to be a primitive member of the genus in view of the fact that communication dances in this species are performed on horizontal plane instead of vertical plane used by domesticated bees. Both on the basis of chromosome number and primitive behaviourism, it is possible that the domesticated tetraploid bees *A. indica* and *A. mellifera* may have evolved from wild diploid forms like *A. florea.*

Polyploidy as a process in speciation is far more common in plants than in animals. Rarity of polyploidy in animals is mainly due to their usual bisexual condition. Incidence of polyploidy among animals is often associated with occurrence of hermaphroditism or parthenogenesis. Parthenogenesis in its turn is usually associated with polyploidy or hybridization or both. The relative evidence on this point has been critically reviewed by Suomalainen (1952). Arhenotoky or generative parthenogenesis with diploid female and haploid male characterises the whole group of *Hymenoptera* from primitive horn tail and saw-flies to highly evolved social ants, wasps and bees. As some of the primitive genera of this order have been found in the upper jurassic strata, Whiting (1945) infers that parthenogenetic habit in *Hymenoptera* may have preceded polyploidy which is comparatively less common in this order.

IX. PHYLOGENETIC INTERRELATIONS IN THE FAMILY OF BEES

Fossil records offer very meagre evidence for tracing the ancestry of bees. On the basis of comparative anatomy, morphology and bionomics, Michener (1954) has formulated a phylogenetic tree indicating possible relationships among subfamilies and tribes of bees. According to his grouping the subfamily *Apidæ* contains the tribes *Apini*, *Meliponini*, *Bombini* and *Euglossini*, whereas the tribe *Xylocopini* is included in the subfamily *Megachilidæ*. Comparison of chromosome numbers indicates that certain *Osmia*, *Xylocopa* and *Euplassia* species have 32 chromosomes in female and 16 in males as in domesticated bees. *Melipona* and *Trigona*, which are closest to bees on many other criteria, show different chromosome number 18 in female and 9 in male. *Bombus* has $2n = 12$ both in males and females (Kerr,

1948) and therefore, not as closely related to *Apis* though these two have been included in a common subfamily by Michener.

Comparative studies on ontogeny as also behaviour patterns particularly relating to construction of nest give valuable clues for tracing the phylogeny of social insects. Thus, for instance, there have been a series of changes in the design of wax combs in primitive to advanced bees. The comb may be (1) an irregular cluster of wax pots or a regular arrangement of hexagonal cells, (2) of undifferentiated or differentiated cells, (3) single or multiple, (4) horizontal or vertical, (5) with cells either on upper or lower or both sides and (6) exposed to light or enclosed in dark. Occurrence and combinations of such homologous characters correlated with geographical distribution may indicate probable or improbable species entering the phylogeny of domesticated *Apis* species. Kerr and Laidlaw (1956) have traced the sequence of changes in comb design starting from ' Pocket makers ' or ' Pollen primers ' among primitive *Bombus* species up to an elaborate comb architecture attained in *A. mellifera*. They have also shown how the exposed ' cluster brood ' types of primitive *Trigona* have ultimately evolved into most advanced types like *Trigona staudingeri* having vertically suspended multiple two-sided combs enclosed in involucrum. It is interesting to trace parallel changes in the genus *Apis*. Primitive *A. florea* and *A. dorsata* have single vertical comb exposed to light while *A. indica* and *A. mellifera* have multiple combs in dark enclosure. Changes from primitive exposed cluster brood type *Trigona* to *Trigona staudingeri* are fairly continuous so that all intergrades actually exist. But the change from single exposed to multiple enclosed comb types in *Apis* is rather abrupt without intermediate connecting links. This change may have been due to a series of adaptive mutations as suspected in the case of *Trigona* (Kerr and Laidlaw, 1956). But such mutations are more likely in diploid forms like *A. florea* rather than in tetraploid *Apis*. Diploid *Apis* species building multiple combs in dark are unknown. Polyploidy may account for quantitative differences in diploid and tetraploid *Apis* in respect of characters like body-size or tongue length. But mere polyploidy by itself cannot explain so conspicuous a qualitative change in respect of an important behaviour pattern such as comb design. Apart from mutational change, it is equally probable that hybridization of primitive diploids like *A. florea* with another very closely related diploid form of *Apis* or an adjacent genus, building multiple combs in dark, followed by chromosome duplication, may have contributed to the origin of domesticated tetraploid bees.

Among all the members of the bee family, the genus *Apis* shows closest affinity with *Trigona* and *Melipona* in spite of their different basic numbers

8 and 9 respectively. *Melipona* and *Trigona* are mainly distributed south of tropics in India, Pacific Islands, Australia, Africa up to and as far as South America (Schwarz, 1948). They may have some common centre of origin from where they could have spread over continental blocks that have now drifted far apart. Distribution of *Apis*, *Trigona* and *Melipona* overlaps in Indo-Malayan regions. If at all there has been any hybridization at diploid level among *Apis* and *Trigona*, such an event was possible near about India where members of both these genera co-exist.

Trigona staudingeri Gribodo from Africa constitutes the best known living link between *Apini* and *Meliponini* (Smith, 1954; Kerr and Laidlaw, 1956). It builds vertically suspended multiple combs enclosed in involucrum. Chromosome number of *Trigona stuadingeri* is not yet reported nor it is known whether it occurs in Indo-Malayan region or whether any other *Trigona* in this area have similar comb design. It would be interesting to investigate the Indian *Trigonas* more critically in these respects.

X. EVOLUTION AMONG *Apis* SPECIES

The foregoing considerations indicate that the domesticated *Apis* species evolved from primitive diploids by (1) polyploidy combined with a series of adaptive mutations for certain characters like multiple enclosed combs, or (2) hybridization at diploid level followed by polyploidy. In view of the close association of the bees with flowering plants, origin of nectar and pollen gathering solitary and social bees must have been co-eval with or subsequent to the origin of *Angiosperms* somewhere around the tertiary period. Fossils of bumble bees have been found in Baltic ambers of lower oligocene (Hatch, 1950). Advent of large-scale polyploidy both among plants and animals has been found to be closely associated with the pleistocene glaciation (Suomalainen, 1952). It is quite likely that origin of tetraploid domesticated bees may have synchronised with this glaciation which extended as far as parts of Northern India and covered it under ice sheets for a long period. It is, therefore, reasonably probable that primitive diploids like *Trigona* and *A. florea* originated and spread over fairly wide areas much before this pleistocene glaciation which seems to mark the origin of tetraploid *A. indica*. During the post-glacial period, *A. indica* may have gradually spread over the Indo-Malayan regions slowly differentiating into races, varieties and subspecies. The northward migration of the primitive tetraploid *A. indica* seems to have followed three main routes along (1) Indo-Burmese Frontiers to China, Japan and North Pacific Islands, (2) Indo-Iranian Frontiers into Arabia, Egypt and Africa and (3) Indo-Caucasian Frontiers into Central Asia, Mediterranean countries to Europe. During

the course of its Trans-Himalayan migration, the primitive tetraploid *A. indica* may have gradually differentiated into an advanced tetraploid *A. mellifera*. All along these three migratory routes, *A. mellifera* differentiated further into a number of African, European and Sino-Japanese races listed earlier. All previous attempts for understanding the course of evolution in bees have been highly speculative and the conjectures presented here have therefore to be of the same category.

Introductions of *A. mellifera* in Australia and America have followed their colonisation. It was introduced in North America in the 17th Century and still later in South America in the 19th Centruy (Lindauer, 1957). Arrival of *A. mellifera* in South America assumes special significance. It brought together the most advanced bee with one of its most primitive ancestors—the *Meliponins* of South America.

General trends in phylogeny and past evolution reasonably indicate that the primitive tetraploid *A. indica* gradually differentiated into *A. mellifera* which may have appeared much later as a taxonomic derivative of the former. The prevalent tendency of grouping all *indica* bees as a subspecies of *A. mellifera* provides a genuine case for our reconsideration.

XI. Discussion and General Remarks

For the purely practical purpose of breeding better strains of Indian bees, all foregoing considerations lead to the following conclusions:—

(1) Since Indo-Malayan region constitutes the centre of origin of bees, wealth of diversity of natural forms provides ample scope for selection.

(2) Both *A. indica* and *A. mellifera* have the same chromosome number so that through their hybridization, it should be possible to combine desirable characters of both. This can include hardiness, disease resistance or tolerance to weather extremes from *A. indica* with body-size, tongue length, prolificity or honey gathering capacity of *A. mellifera*.

(3) For this purpose, hybridization of *A. indica* with nearer ecotypes of *A. mellifera* like *caucasica*, *ligustica* or *sinensis* may give better results than the distant ecotypes like *silvarum* or *japonica* which must have undergone extreme genic differentiation.

(4) Breeding technique and procedure has to be appropriately related to the tetraploid constitution of these bees.

(5) Arhenotoky in bees with $2n$ females and n males leads to peculiar genetic effects. The recessive genes with deleterious effects in such natural populations may be retained through $2n$ females but drastically eliminated

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through haploid males at each generation. Such elimination would be far greater in diploid species like *A. florea* but comparatively much less in *A. indica* or *A. mellifera* in view of their polyploid constitution.

(6) Progressive homozygosis in such species has undesirable effects on sex mechanism, brood viability and vigour. Therefore, inbreeding, which is a routine procedure in breeding other organisms, has serious limitations in bees.

(7) Selection of superior genotypes, securing their intercrossing under isolation and testing the performances of progenies, constitutes a workable procedure for routine bee breeding.

Work on breeding better strains of Indian bees, which is in progress at this laboratory, shows that the range of natural diversity in respect of many economic attributes such as body-size, tongue length, prolificity or honey gathering capacity is quite considerable and the upper limits in these ranges come fairly close to the reported averages in *A. mellifera*.

XII. SUMMARY

Normal somatic complement in *A. indica* consists of 32 chromosomes in female and 16 in male. Karyo-typic analysis of haploid males shows eight homomorphic pairs indicating distinct somatic association (2 *m.c.* + 2 *s.m.c.* + 4 *s.t.c.*).

Excepting the gonial and epithelial layers of blastoderm, other tissues show polysomatic increase in euploid or aneuploid numbers through repeated endomitosis.

In view of occurrence of eight homomorphic pairs in the male showing somatic association, it is inferred that the male, though numerically haploid is genetically diploid thus the famale being a tetraploid. This is supported by control of certain characters by multiple genes without linkage and diploid, constitution of *A. florea* having 16 chromosomes in female and 8 in male.

It is suggested that *A. indica* originated from primitive diploid by (1) polyploidy combined with adaptive mutations for multiple enclosed comb habits or (2) hybridization at diploid level among closely related *Apis* and *Trigona* followed by polyploidy during pleistocene glaciation in the Indo-Malayan region.

Primitive *A. indica* gradually evolved into *A. mellifera* which differentiated further into a number of African, Eurasian and Sino-Japanese races during the course of the passage through the three main northern migratory routes of the Himalayas.

Bearings of these observations on the practical problems of breeding better strains of Indian bees are discussed.

Investigations on comparative cyto-genetics of Indian species of *Apis*, *Trigona* and related genera are in progress.

XIII. Acknowledgements

Authors are thankful to Shri S. G. Shende for providing certain facilities, Shri R. P. Phadke for photographs and Shri S. R. Salvi for valuable help rendered in the laboratory.

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XV. EXPLANATION OF PLATE III

FIG. 1. Camera lucida drawing of somatic complement in the female showing 32 chromosomes, × 3,237 Approx. (*a*) Photomicrograph of Fig. 1, × 875. (*b*) Photomicrograph of Fig. 1, × 1,750.

FIG. 2. Camera lucida drawing of somatic complement in the male showing 16 chromosomes somatically associated in pairs, × 3,237 Approx. (*a*) Photomicrograph of Fig. 2, × 875. (*b*) Photomicrograph of Fig. 2, ×1,750. (*c*) Photomicrograph of Fig. 2, × 2,725.

FIG. 3. Idiogram of the somatic complement in the male representing 8 homomorphic pairs.

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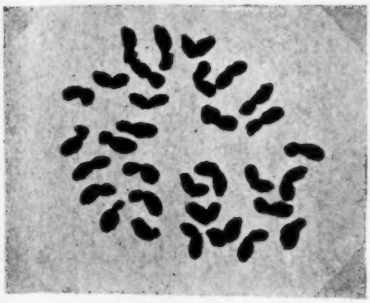

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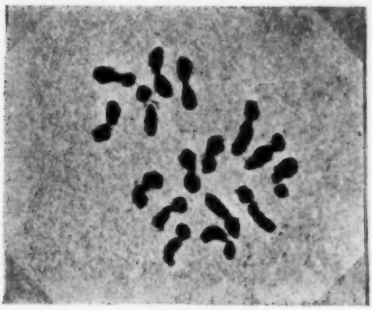

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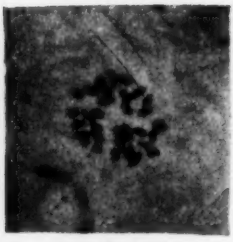

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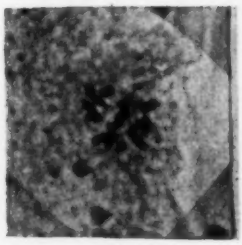

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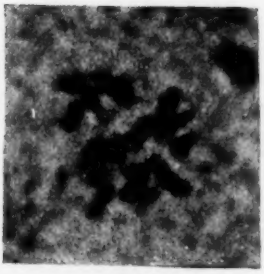

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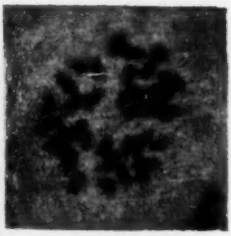

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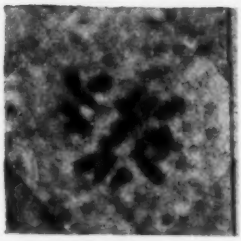

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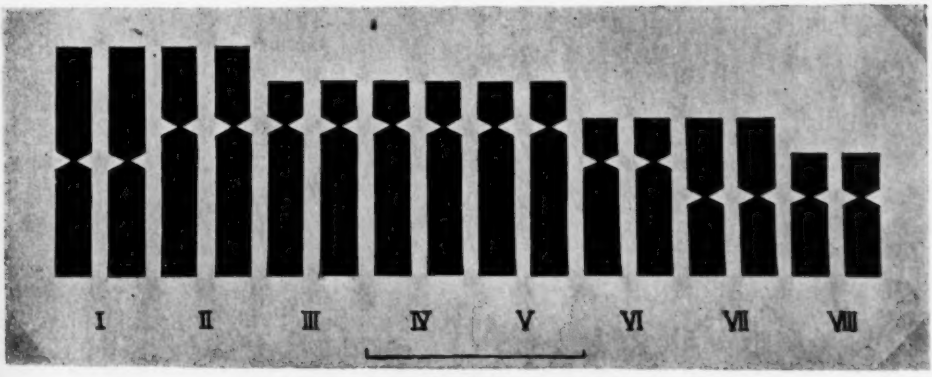

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FIGS. 1-3

STUDIES ON THE NUTRITION OF RICE PLANT (*ORYZA SATIVA* L.)

Part III. *Partial Efficiency* of Nitrogen Absorbed by Rice Plant at Different Stages of Growth in Relation to Yield of Rice (*O. sativa* var. *indica*)

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(*Central Rice Research Institute, Cuttack*)

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(Communicated by Mr. R. L. M. Ghose, F.A.SC.)

INTRODUCTION

EFFICIENT utilization of nitrogen by rice plant is one of the important factors determining growth and yield of the crop. Tanaka *et al.* (1958 *a*, *b*) have shown that *indica* rice takes up increasing amounts of nitrogen with increase in nitrogen supply but at high manuring levels, the assimilation of nitrogen does not keep pace with nitrogen uptake, with the result that more soluble nitrogen accumulation takes place in the plant body, pulling down yield of grain. This malnutrition stage for *indica* rice at high nitrogen supply was shown to set in at the reproductive stage. It may therefore be expected that nitrogen absorbed by rice plant at certain stages of growth is more efficiently utilized than at other stages, in the formation of growth characters, which ultimately determine the grain yield of the crop.

This problem of effective use of the nitrogen, taken up by the plant at different stages of growth, is best studied under solution culture technique, free from extraneous soil influence. Gericke (1930) was the first to suggest a technique for determining mineral nutrient requirements of the rice plant and to differentiate the total quantities of nutrients taken up by the plant, into a fraction that caused growth and the one that did not. Following his work, qualitative investigations of problem with respect to rice were undertaken by Ishizuka (1933), Osugi and Aoki (1938) and Kasugai (1939). Later, Kimura and Chiba (1943), quoted by Mitsui (1955), proposed a method to express *partial efficiency* of nitrogen absorbed by plant as a measure of utilization of absorbed nitrogen for production of grain. The *partial efficiency* of nitrogen absorbed by plant at different stages of growth was defined by these authors as increase in yield of grain or straw for unit increase in nitrogen absorbed by the plant. Kimura and Chiba (*l.c.*), working with *japonica* rice varieties obtained two periods of maximum *partial efficiency* with low levels of nitrogen supply, first occurring at the early stages of growth and the second sometime before flowering.

It has been earlier shown by Tanaka *et al.* (1958 *b*), that growth character of *indica* variety is different from that of *japonica* variety and that they also differ in nitrogen metabolism at high nitrogen levels. It was therefore considered necessary to study the problem of utilization of nitrogen absorbed at different stages of growth by an *indica* variety of rice, in order to evaluate growth stages of plant, during which nitrogen uptake will give beneficial results for production of grain.

Material and Methods

Experiment was conducted in water culture technique, using a medium duration variety T 141 of 145 days duration. The composition of culture solution was same as used earlier by Tanaka *et al.* (1958 *a*). Pots employed in the present study were however of four litres capacity; two seedlings were grown per pot and culture solution was changed every fifth day. Nitrogen levels of 10 and 60 p.p.m. respectively were used for growing plants. 10 p.p.m. nitrogen is considered the lowest critical limit to maintain plant growth and 60 p.p.m. is considered the upper critical limit, called the luxury utilization limit (Tanaka *et al.*, *a*, *l.c.*). Plan of experiment is shown in Fig. 1. Each treatment step shows the period after which further nitrogen supply to plant was withheld and the plants were allowed to grow on nitrogen absorbed up to that stage. Other nutrients were supplied as usual. Ten such nitrogen treatment steps, inclusive of no nitrogen treatment, each at an interval of ten days were arranged till grain setting and filling was complete. With two nitrogen levels of 10 and 60 p.p.m. and ten periods of nitrogen application, there were in all twenty treatments, each of which was duplicated.

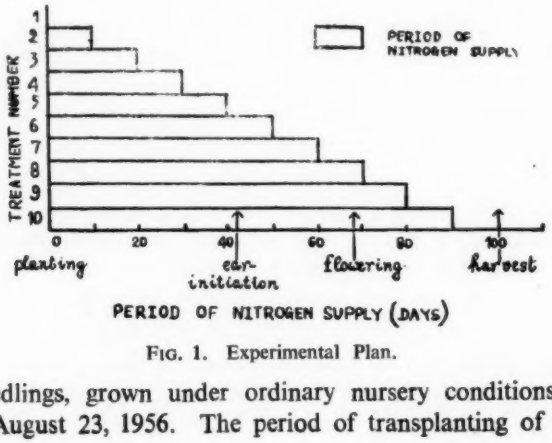

FIG. 1. Experimental Plan.

Rice seedlings, grown under ordinary nursery conditions were transplanted on August 23, 1956. The period of transplanting of the crop was

however about fifteen to twenty days later than the normal transplanting period for the variety. Crop was harvested on December 7, 1956 and observations on top length, root length, panicle number and air-dry weight of ear, straw and root were recorded. Data on set grains per panicle and 1,000 grain weight, etc., for different treatments were also taken. Nitrogen estimation was done on air-dry samples of the plant and nitrogen absorbed as mg./pot was calculated on total dry weight basis. *Partial efficiency* of nitrogen absorbed at successive stages was calculated as follows:

Partial efficiency of nitrogen absorbed during n-th treatment stage after planting is calculated as:

$$\frac{x_n - x_{n-1}}{y_n - y_{n-1}}$$

where x_n and y_n are grain yield and total nitrogen absorbed respectively at n-th treatment stage and x_{n-1} and y_{n-1} are the same characters respectively at the $n-1$-th stage.

Data presented in Tables I to IV with respect to 10 and 60 p.p.m. nitrogen have been designated as 'low' (L) and 'high' (H) series respectively

TABLE I

Dry matter weight, nitrogen per cent. and total nitrogen absorbed

(L series—10 p.p.m. nitrogen)

	L_0	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9
Dry matter weight gm./pot:										
Ear	1·5	2·0	3·8	5·5	10·5	13·3	14·3	17·6	20·1	21·1
Straw	1·8	3·5	6·2	10·0	13·0	16·0	18·0	19·5	20·0	21·0
Root	0·7	1·0	3·0	3·0	5·0	6·0	5·0	4·5	4·5	4·5
Grain weight gm./pot ..	1·5	1·9	3·7	5·3	10·1	12·3	13·1	16·3	19·6	19·9
Total N per cent.:										
Ear	0·93	0·89	0·87	0·90	0·98	1·03	1·06	1·08	1·08	1·17
Straw	0·42	0·36	0·41	0·38	0·39	0·41	0·52	0·61	0·67	0·70
Root	0·52	0·50	0·47	0·52	0·50	0·47	0·51	0·56	0·61	0·60
Nitrogen absorbed mg./pot ..	26	36	71	104	179	232	272	334	379	421
Partial grain weight $(x_n - x_{n-1})$	0·4	1·8	1·6	4·8	2·2	0·8	3·2	3·3	0·3	
Partial N absorbed mg. $(y_n - y_{n-1})$	10	41	33	75	53	40	62	45	42	
Partial efficiency $\frac{x_n - x_{n-1}}{y_n - y_{n-1}} \times 1000$	40	44	48	64	42	20	52	73	7	

and periods of nitrogen supply under each series are represented as $L_0 - L_9$ and $H_0 - H_9$ respectively.

RESULTS AND DISCUSSION

(*a*) *Yield, nitrogen content and* partial efficiency.—Tables I and II show the dry matter weight and nitrogen content of different nitrogen treatments for L and H series respectively at harvest.

It is seen that in the case of L series, grain yield increases with increase in period of nitrogen supply. On the other hand, in the H series, grain yield reaches its maximum at H_6 stage and any longer period of nitrogen supply causes depression in yield. Straw yield in both the series, however, continues to increase with elongation in the period of nitrogen supply.

Nitrogen per cent. in ear, straw and root of the plants in L series increases gradually with elongation in the period of nitrogen supply. In H series also, increase in nitrogen per cent. in plant is gradual up to H_4 stage; but

TABLE II

Dry matter weight, nitrogen per cent. and total nitrogen absorbed

(H series—60 p.p.m. nitrogen)

	H_0	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
Dry matter weight gm./pot										
Ear	1·5	2·4	5·6	14·1	21·7	25·6	27·2	27·0	23·9	20·6
Straw	1·8	3·3	7·2	19·0	29·0	37·5	42·0	45·0	46·0	46·2
Root	0·7	0·9	2·0	5·0	6·5	7·0	7·5	7·0	7·5	7·4
Grain weight gm./pot ..	1·5	2·1	5·1	13·2	20·5	20·7	20·6	18·4	16·7	12·4
Total N per cent.										
Ear	0·93	0·89	0·81	0·90	1·08	1·30	1·47	1·83	1·88	2·0
Straw	0·42	0·42	0·42	0·45	0·48	0·65	0·85	1·27	1·77	2·1
Root	0·52	0·57	0·52	0·61	0·70	0·73	0·99	1·08	1·60	2·0
Nitrogen absorbed mg./pot ..	26	40	85	244	419	632	831	1142	1383	1510
Partial grain weight ($x_n - x_{n-1}$)	0·6	3·0	8·1	7·3	0·2	−0·1	−2·2	−1·7	−3·8	
Partial N absorbed mg. ($y_n - y_{n-1}$)	14	45	159	175	213	199	311	241	190	
Partial efficiency $\frac{x_n - x_{n-1}}{y_n - y_{n-1}} \times 1000$	43	67	57	42	0·9	−0·5	−7	−7	−20	

at later stages, there is a marked increase in nitrogen content in all parts of the plant body. Nitrogen content is particularly high in straw and root after H_7 stage and later, where depression in grain yield sets in.

Partial efficiency of nitrogen absorbed at successive stages of nitrogen supply for production of grain has been worked out and is shown in Tables I and II and represented graphically in Fig. 2. The curve for L series shows two peaks of *partial efficiency*. The first peak for efficient utilization of nitrogen in the L series occurs during the vegetative stage, about thirty-five days before flowering and the second peak occurs at about the flowering stage. In H series, on the other hand, only one peak period of *partial efficiency* occurs at the early stages of development, about fifty days before flowering. The results obtained are in general agreement with those reported by Kimura and Chiba (1943), for a *japonica* variety of rice. They, however, reported that at medium supply of nitrogen (2·0 to 12·4 p.p.m.), the second peak period of *partial efficiency* in the *japonica* variety occurred about fourteen days before flowering and was not affected by variation in nitrogen supply. The second peak of *partial efficiency* in the *indica* variety, examined in the present study, however, occurred at about the flowering stage.

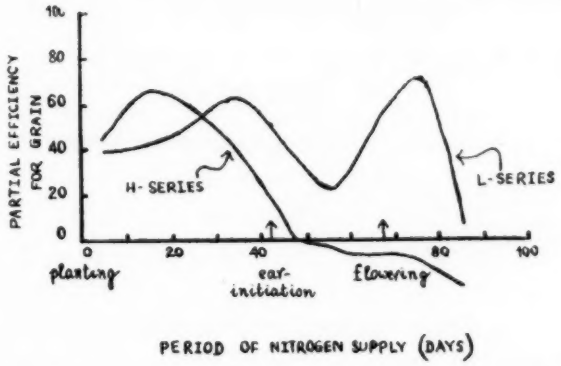

FIG. 2. *Partial efficiency* of nitrogen absorbed at different stages of growth.

(*b*) *Yield components.*—Data on panicle number per pot, mean weight and length of panicle, number of filled grains per panicle and 1,000 grain weight of harvest material, both for L and H series respectively are shown in Tables III and IV.

At low nitrogen supply (L series, 10 p.p.m.), where nitrogen given is just sufficient to maintain growth, stoppage of nitrogen at any stage showed nitrogen deficiency and resulted in lower yield of grain. Effect of nitrogen

TABLE III

Characters of yield components at harvest

(L series—10 p.p.m. nitrogen)

	L_0	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9
Panicle No./pot	2·0	2·0	3·5	5·0	9·0	10·5	10·5	11·0	11·0	11·0
Mean panicle weight gm. ..	0·77	1·00	1·10	1·10	1·16	1·27	1·37	1·60	1·83	1·92
Mean panicle length cm. ..	13	15	15	15	16	17	18	20	20	20
Mean weight/panicle gm.:										
Chaff	0·00	0·00	0·00	0·00	0·00	0·02	0·05	0·07	0·00	0·00
Filled grain	0·75	0·95	1·05	1·06	1·13	1·17	1·25	1·48	1·78	1·81
Weight of 1,000 grain gm. ..	18·4	18·9	20·2	20·0	19·9	18·6	18·0	18·2	20·9	21·2
Number of filled grains per panicle	41	50	52	53	57	63	69	80	85	85

TABLE IV

Characters of yield components at harvest

(H series—60 p.p.m. nitrogen)

	H_0	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
Panicle No./pot	2·0	3·5	5·5	12·5	16·0	23·5	26·5	27·5	27·0	28·0
Mean panicle weight gm. ..	0·77	0·69	1·02	1·13	1·36	1·09	1·03	0·98	0·88	0·78
Mean panicle length cm. ..	13	13	15	16	19	20	20	20	19	19
Mean weight/panicle gm.:										
Chaff	0·00	0·04	0·01	0·02	0·04	0·13	0·16	0·19	0·21	0·21
Filled grain	0·75	0·61	0·93	1·06	1·28	0·88	0·78	0·67	0·62	0·46
Weight of 1,000 grain gm. ..	18·4	17·5	19·6	18·5	18·6	14·5	14·9	14·2	14·5	13·3
Number of filled grains per panicle	41	35	47	57	68	61	52	50	50	34

taken up by plant at various stages of growth on different yield components is shown in Fig. 3. In the figure, yield of grain per pot and various yield components, such as number of panicles per pot, panicle weight, number of filled grains per panicle and 1,000 grain weight, obtained under the L series are plotted against different stages of nitrogen supply. It is seen that grain yield increases with elongation in the period of nitrogen supply from L_1 to L_9 stage. Various yield components which collectively determine yield of grain are, however, affected differently with elongation in the period of nitrogen supply. Panicle number per plant increases from L_1 stage to L_4

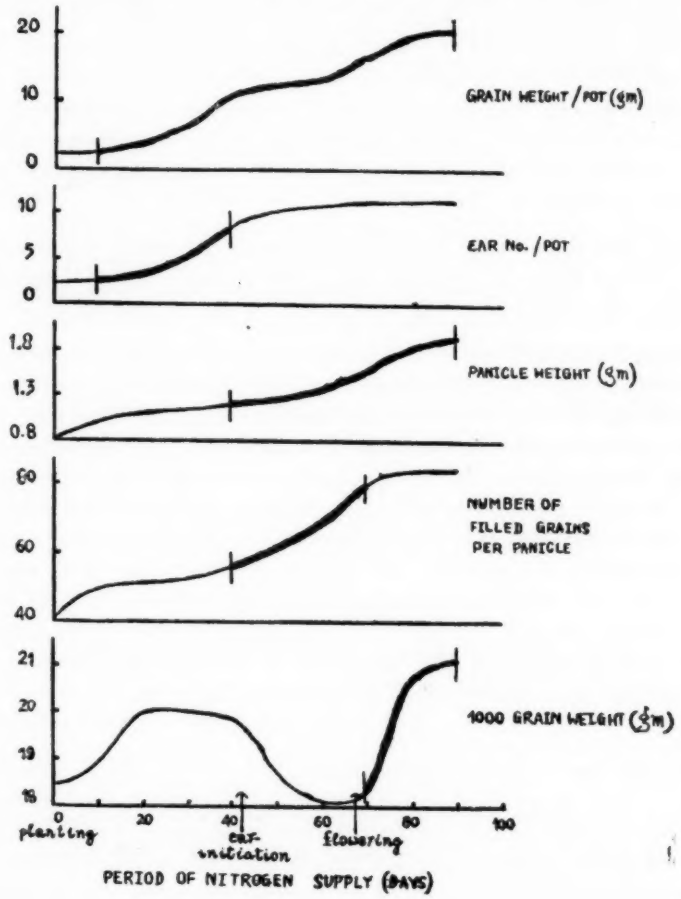

FIG. 3. Influence of time of nitrogen application on different yield components.

stage and panicle weight increases from L_4 stage to L_9 stage. Number of filled grains per panicle increase from L_4 stage to L_7 stage and effective 1000 grain weight increases from L_7 stage to L_9 stage.

At high nitrogen supply (H series, 60 p.p.m.), however, panicle number per pot increases markedly up to H_6 stage and later, there is very little change. Panicle weight, number of filled grains per panicle and 1,000 grain weight in the H series, all attain maximum value at nitrogen supply up to H_4 stage (the ear-initiation stage) and decrease later with elongation in the period of nitrogen supply. Depression in all these yield components is accompanied with marked increase in chaffiness of the panicle, which is also a contributing factor in decreasing yield with elongation in the period of nitrogen supply after H_6 stage.

H series plants, with continuation of nitrogen supply after H_4 stage, also suffered from neck infection due to blast (*Piricularia oryzæ*). Observations on neck infection due to blast taken at milk stage of November 14, 1956 are shown in Table V.

It was seen that, while L series plants were completely free from neck infection due to blast, the H series plants were heavily affected, particularly at H_6 stage and after, where the depression in grain yield occurred.

The results show that a rice variety of medium duration (about 145 days) shows, during its growth phase, two stages, where nitrogen taken up by the plant is efficiently utilized for production of grain. One such growth period is at the vegetative stage and the second at the flowering stage. When the plant is grown at low level of nitrogen supply, just enough to maintain growth, nitrogen absorbed at these stages is effectively utilized and any lack of nitrogen occurring at these stages is likely to affect production of grain seriously. If, however, the plant is grown at high manuring level

TABLE V

Neck infection due to blast (Piricularia oryzæ)

(Number of infected panicles per pot)

Stages of N application	0	1	2	3	4	5	6	7	8	9
L series (10 p.p.m. N)	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
H series (60 p.p.m. N)	0·0	0·0	0·0	0·0	1·5	4·5	6·0	5·5	6·0	6·0

from the start, it is capable of storing nitrogen in the plant tissue for later growth and lack of nitrogen at later stages at flowering will not affect the grain yield; on the contrary, a high nitrogen supply at flowering under these conditions will seriously pull down grain yield. This malnutrition condition due to high accumulation of nitrogen has been attributed by Tanaka *et al.* (1958 *a*) to accumulation of comparatively more soluble nitrogen in the plant body, resulting in increased yield of straw and reduction in yield of grain.

It is also seen that under moderate manuring, nitrogen taken up by rice plant at various stages plays a definite role in the development of plant characters which determine the yield of crop. During vegetative stage, nitrogen uptake increases tillering capacity of the plant and determines effective panicle number. From ear-initiation to flowering, nitrogen plays a role in increasing number of filled grains per panicle and from flowering to maturity, nitrogen uptake increases effective 1,000 grain weight. The second stage of nitrogen uptake at the reproductive stage seems therefore to be important from the point of view of increasing production, as nitrogen taken up at this period appears to be utilized properly in the development of grain. Ozaki and Mitsui (1950), quoted by Mitsui (1955), using heavy nitrogen N^{15} as tracer, have also observed that nitrogen applied 20 days before flowering, accumulated fifteen days later, mainly in the boot leaf-blade, culm and ear and was principally utilized in promoting the activities of these organs. Split application of nitrogenous fertilizer, under field condition, first dose given basal or at early stage of vegetative growth and the second dose top-dressed at the reproductive stage, therefore, appears a more rational practice of increasing rice yield than full fertilizer dose given at one time during the vegetative stage.

Summary

Rice plant of medium duration, grown under low nitrogen supply (10 p.p.m.), shows two peak periods of *partial efficiency* for nitrogen, when nitrogen taken up by the plant is most efficiently utilized in increasing grain yield. First such period occurs at the tillering stage and the second at flowering, between the boot and milk stages.

Nitrogen taken up by the plant between tillering and ear-initiation plays role in increasing tiller and panicle number per plant. During panicle development, from ear-initiation to flowering, nitrogen taken up increases number of filled grains per panicle and, after flowering, it increases effective 1,000 grain weight. The first period of maximum *partial efficiency* is, therefore, for increasing tiller number and the second for increase of panicle weight.

If, however, the plant is grown at high levels of nitrogen (60 p.p.m.) from the start, nitrogen taken up during the vegetative stage, up to ear-initiation, is stored in the body of the plant and is used in the later growth and development, even if nitrogen supply is cut off later. High nitrogen supply, after ear-initiation, has detrimental effect on growth and decreases grain yield because of decrease in number of filled grains per panicle and 1,000 grain weight. Chaffiness of panicle, under this condition, also markedly increases and plant becomes more susceptible to neck infection due to blast.

The authors are indebted to Mr. R. L. M. Ghose, Director, Central Rice Research Institute, Cuttack, for suggestions and improvement in the text of the paper.

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STUDIES ON THE NUTRITION OF RICE PLANT (*ORYZA SATIVA* L.)

Part IV. Growth and Nitrogen Uptake of Rice Varieties (*O. sativa* var. *indica*) of Different Durations

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(Communicated by Mr. R. L. M. Ghose, F.A.SC.)

Rice varieties of different durations have been known to exhibit varietal differences in their growth and development. Ramiah and Narasimham (1936) stated that in early duration varieties, ear-initiation closely followed maximum tillering, while in case of medium and late duration varieties, ear-initiation occurred sometime after maximum tillering stage was reached. Process of nitrogen uptake of different duration *indica* varieties in relation to their growth has, however, not been studied. Only information available on this subject is with respect to *japonica* variety of rices (Ishizuka and Tanaka, 1952; Takahashi *et al.*, 1955 and Maeda and Murakami, 1956), which are comparable only with some early duration *indica* varieties.

Tanaka *et al.* (1959), conducting water culture studies, have reported that rice plant, during its growth, shows two peak periods of nitrogen efficiency, one at tillering stage and another at ear development stage, when nitrogen taken up by the plant is most efficiently utilized for increasing grain yield. From this, it may be assumed that rice crop, grown under field conditions, will also absorb nitrogen vigorously at these two stages of growth. As rice varieties of different durations have been known to show differences in their growth character, it was thought that there may also be differences in the process of nitrogen uptake in relation to their growth. Investigations were, therefore, undertaken to study the process of nitrogen uptake during the growth and development of some *indica* rice varieties of early, medium and late durations, grown under field condition, in order to know the exact growth stages, when nitrogen requirements of different varieties were high. It was considered that this study may throw light on manurial practices to be adopted with respect to different duration rice varieties, for obtaining optimum yield.

MATERIAL AND METHODS

Early duration varieties, Ptb 10 (110 days) and MTU 15 (130 days), medium duration variety T 141 (150 days) and late duration variety BAM 9 (165 days) were grown under field condition for this study. Varieties were sown on June 16, 1956 in seedbeds, which received a basal dressing of eleven cartloads of compost per hactare. Seedbeds were also topdressed with ammonium sulphate at 10 kg. N per hactare on July 9, 1956. Transplanting of all the varieties was done on July 20, 1956 and about two to three seedlings were planted per hill. Spacing adopted was 25×15 cm. for early duration varieties and 25×25 cm. for medium and late duration varieties. Before transplanting, tops of the seedlings were cut to the extent of 15–20 cm. This is a common practice, adopted for all varieties in this tract. Main fields were manured with 30 kg./ha. each of nitrogen, phosphate and potash, in the form of ammonium sulphate, superphosphate and potassium sulphate respectively.

Early duration varieties were grown at higher locations, medium duration varieties in medium lands and late duration varieties were grown in low-lying fields. This is the usual practice adopted for growing different duration varieties under rainfed conditions, as water in medium and low-lying areas remain for a longer period after cessation of monsoon and such lands are, therefore, fit for growing late maturing varieties only.

Plant samples for study and analysis were taken every 10 days after sowing. At the nursery stage, each sample consisted of 200–100 plants and at the main field stage, it consisted of ten hills, collected at random. Sampled plants were washed thoroughly, free from adhering soil, root portions were discarded and after recording plant height and tiller number and fresh weight, these were cut into small pieces for determination of dry matter per cent. For this, a weighed quantity of representative material was dried in an oven at 70–80° C. for twenty-four hours. At flowering stage, straw and earheads from each sample were separately weighed and dried.

Nitrogen estimation in samples was done by Microkjeldahl method, after powdering the dried material through a Willey grinding mill.

GENERAL CONDITION OF PLANT GROWTH

All varieties put up normal growth up to flowering. Variety BAM 9, however, lodged badly before flowering and was, therefore, propped by bamboo supports.

RESULTS AND DISCUSSION

1. *Growth process.*—Growth process of all the varieties, expressed as mean plant height and tiller number per hill is shown in Fig. 1. Ear-

initiation stage of each variety is also indicated in the figure. It is seen that increase in top length of all the varieties follows, more or less, a same pattern and the later duration varieties put up higher top length than the earlier duration ones. Tiller number for all varieties continues to increase up to

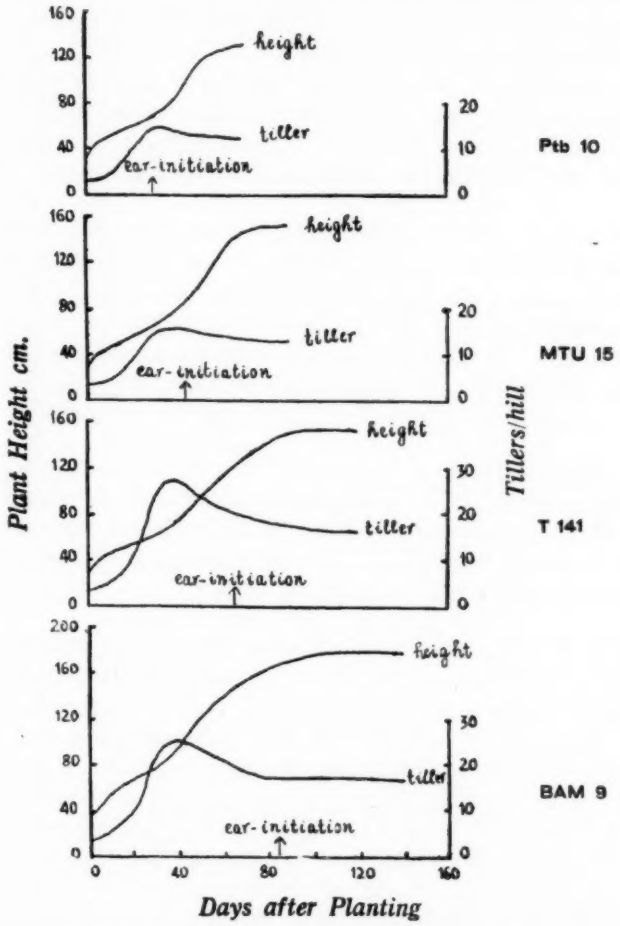

FIG. 1. Growth process of different rice varieties.

a certain stage and after attaining a maximum, it decreases due to death of late and ill-developed tillers. The maximum tiller number stage for all the varieties is obtained, more or less, at the same period of growth, about thirty to forty days after transplanting, irrespective of the duration of the varieties. Time of ear-initiation, which marks the end of vegetative stage and beginning of reproductive stage, however, is seen to vary with different duration

varieties. In the case of early duration variety Ptb 10, ear-initiation closely follows the maximum tiller number stage. With MTU 15, ear-initiation occurs about 10 days after the maximum tillering stage and with medium and late duration varieties, T 141 and BAM 9, ear-initiation occurs thirty and forty-five days respectively, after maximum tillering stage. Results show that in early duration varieties, formation of ear primordia, immediately follows the maximum tiller number stage, while in the later duration varieties, there is a time lag between maximum tillering stage and beginning of reproductive growth, the interval increasing with increase in the duration of the variety. These observations are in agreement with those reported earlier by Ramiah and Narasimham (1936) and Grant and Thien Aung (1941).

Observations on the emergence of leaf on the main shoot were also taken and it was seen that the varieties differed in the number of leaves they put up before flowering. Early duration varieties Ptb 10 and MTU 15 flowered at twelfth and thirteenth leaf stage respectively, while the medium and late duration varieties T 141 and BAM 9 flowered at eighteenth and nineteenth leaf stages respectively. Maximum tiller number for all the varieties was, however, obtained at 12–13 leaf stage, irrespective of duration of variety.

2. *Nitrogen uptake*.—Absorption process of nitrogen of different varieties during growth, from transplanting to maturity is shown in Fig. 2. Nitrogen uptake is expressed as mg. per hill and is calculated from dry matter weight and nitrogen per cent. in plant at different stages of growth. This latter data for all varieties are given in Table I.

In early duration varieties, Ptb 10 and MTU 15, the process of nitrogen absorption is a simple sigmoidal curve. It is seen that just after transplanting, the absorption of nitrogen is rather slow, but with the establishment of seedlings, about ten to twelve days after transplanting, the absorption process becomes vigorous and after attaining a maximum value, it again slows down with flowering. This process of nitrogen uptake of early duration varieties is the same as observed for *japonica* varieties by Ishizuka and Tanaka (1952), Takahashi *et al.* (1955) and Maeda and Murakami (1956).

The absorption process of medium and late duration varieties is, however, different. The uptake of nitrogen at early stages of growth is very vigorous and is of similar nature as in the early duration varieties. This uptake slows down after the attainment of maximum tiller number stage and continues to be slow till ear-initiation stage is reached. At this stage, another period of vigorous nitrogen uptake is obtained which lasts till the

flowering stage. With attainment of flowering, there occurs a nitrogen loss, probably because of flow back of nitrogen from aerial parts.

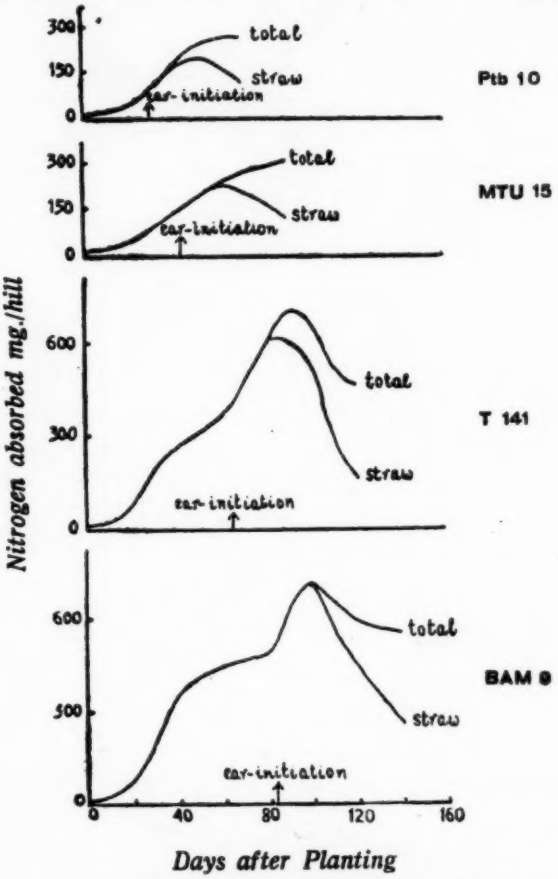

FIG. 2. Nitrogen uptake of different rice varieties.

Rate of nitrogen absorption at different stages of growth for all varieties (taking the highest rate of absorption of each variety as 100) is shown in Fig. 3. This rate has been calculated as the amount of nitrogen absorbed per day for each ten days interval of growth between two sampling dates. Each variety shows two peaks of maximum nitrogen absorption and these peaks, for all the varieties, are seen to correspond to two peaks of *partial efficiency* of nitrogen, described earlier by Tanaka *et al.* (1959). The first peak period of nitrogen absorption in all varieties occurs about thirty to thirty-five days after transplanting and is related to maximum tiller number

TABLE I

Dry matter weight and nitrogen content of different duration rice varieties from transplanting to maturity

Variety and plant characters		Days after planting														
		0	10	20	30	40	50	60	70	80	90	100	110	120	130	140
Ptb 10 (110 days)																
Dry matter gm./hill	Straw	0·20	0·50	1·60	5·10	9·70	15·20	18·40	14·70	..	..	..	..	..	..	..
	Ear	..	..	..	..	..	2·10	6·70	13·50	..	..	..	..	..	..	..
N %	Straw	1·24	2·17	2·52	1·99	1·57	1·25	0·95	0·76	..	..	..	..	..	..	..
	Ear	..	..	..	..	..	1·11	1·18	1·12	..	..	..	..	..	..	..
MTU 15 (130 days)																
Dry matter gm./hill	Straw	0·20	0·60	1·70	4·70	8·60	13·70	24·90	28·90	25·90	20·40	..	..	..	..	..
	Ear	..	..	..	..	..	..	..	4·60	14·00	16·90	..	..	..	..	..
N %	Straw	1·17	2·27	2·57	1·95	1·67	1·35	0·92	0·72	0·63	0·63	..	..	..	..	..
	Ear	..	..	..	..	..	..	..	1·03	0·97	1·15	..	..	..	..	..
T 141 (150 days)																
Dry matter gm./hill	Straw	0·20	0·80	2·30	8·40	15·60	24·40	36·20	53·40	68·70	72·20	67·50	62·90	41·10	..	..
	Ear	..	..	..	..	..	..	..	..	..	6·80	10·60	22·10	30·90	..	..
N %	Straw	1·24	2·81	3·18	2·52	1·82	1·33	0·99	0·92	0·89	0·85	0·80	0·44	0·37	..	..
	Ear	..	..	..	..	..	..	..	..	..	1·52	1·30	1·00	1·03	..	..
BAM 9 (165 days)		..														
Dry matter gm./hill	Straw	0·30	0·90	3·10	8·50	18·60	30·60	42·60	47·60	48·50	55·0	70·00	72·50	68·10	51·80	47·20
	Ear	..	..	..	..	..	..	..	..	..	..	..	6·00	14·00	21·50	28·10
N %	Straw	1·17	2·96	3·18	2·51	1·99	1·40	1·06	1·00	1·00	1·20	1·04	0·78	0·64	0·65	0·53
	Ear	..	..	..	..	..	..	..	..	..	..	..	1·05	1·12	1·07	1·04

stage, which occurs in all varieties almost at the same time. The second peak period of nitrogen absorption is related to ear development. In early duration varieties Ptb 10 and MTU 15, it immediately follows the first peak,

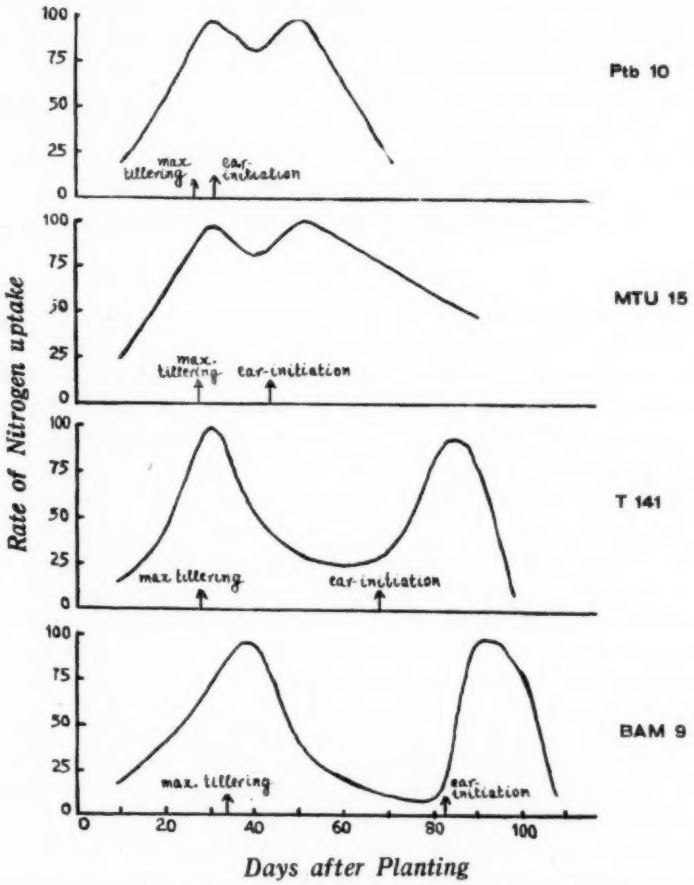

FIG. 3. Rate of nitrogen uptake of different rice varieties.

while in the medium and late duration varieties, it occurs about forty-five to fifty days after maximum tiller number stage is reached. Nitrogen uptake in early duration varieties is, therefore, a continuous process, while in the medium and long duration varieties, two distinct phases of vigorous nitrogen uptake occur, one at the maximum tillering stage and the second at ear development stage.

Changes in the nitrogen content of plant (expressed as per cent. of dry matter) during the seedling stage is shown in Table II. Similar data from planting to maturity are given in Table I. It is seen that during germination

TABLE II

Nitrogen content of rice varieties at the seedling stage of growth

Variety	Duration (days)	N Per cent., days after sowing		
		10	20	30
Ptb 10	110	2·77	1·68	1·46
MTU 15	130	2·63	1·73	1·39
T 141	150	2·77	1·83	1·59
BAM 9	165	2·40	1·80	1·53

in the seedbed stage, there is a common trend in all the varieties and nitrogen per cent. in seedlings drops with growth in the seedbed. After transplanting in the main field, however, nitrogen per cent. in the plant continues to increase for about twenty days. The highest nitrogen content attained in the early duration variety is about 2·5 per cent. and that in the medium and late duration varieties is about 3·2 per cent. After reaching maximum, nitrogen content in all varieties shows a downward trend and at harvest stage, nitrogen content, particularly of straw, is generally less for medium and long duration varieties than for the early duration varieties.

Nitrogen per cent. in all varieties at the maximum tillering stage is seen to be about 1·7 to 2·0 per cent. At later stages of growth, decrease in nitrogen per cent. of plant is associated with decrease in tiller number per hill. At ear-initiation stage, however, nitrogen per cent. in the plant varies from variety to variety, being less in longer duration varieties. Changes in nitrogen content of early duration varieties Ptb 10 and MTU 15 are almost the same as reported for *japonica* varieties by Takahashi *et al.* (1955) and Maeda and Murakami (1956).

These investigations show the characteristic relationship between growth and nitrogen uptake by rice plant. Nitrogen requirement of rice plant is seen to be high at the maximum tillering stage and again at ear development stage. Results have a practical significance under field manuring conditions. In water-logged rice soils, nitrogen losses are known to occur due to

denitrification and drainage (Abichandani and Patnaik, 1958; De and Digar, 1954 and 1955). These factors may, sooner or later, deplete the soil of applied nitrogen. Full dose of fertilizer nitrogen, applied to soil at early stages of growth may, therefore, not be equally effective in increasing crop yield and need for second application at ear development stage may arise. Split application of fertilizer, first application basal at planting and second top-dressed at ear-initiation stage, therefore, appears desirable to assure adequate supply of nitrogen for uptake by crop for growth and development. Split application of fertilizer is likely to be more beneficial in the case of medium and long duration varieties, because of longer time lag between maximum tillering stage and ear primordia formation.

Summary

Different duration varieties Ptb 10 and MTU 15 (early duration), T 141 (medium duration) and BAM 9 (late duration), grown under field condition, have been examined for their growth and process of nitrogen uptake. Following characteristics are observed:—

1. Maximum tiller number in all the varieties is reached at about thirty to forty days after transplanting, irrespective of duration of the varieties. At this stage, all varieties reach twelve to thirteen leaf emergence period on the main shoot. Nitrogen content of the plant at the maximum tiller number stage is about 1·7 to 2·0 per cent. in all the varieties.

2. In early duration varieties Ptb 10 and MTU 15, development of ear primordia takes place at or immediately after maximum tiller number stage, whereas in the case of medium and long duration varieties T 141 and BAM 9, ear primordia develops about thirty and forty-five days respectively, after maximum tiller number stage is reached. Nitrogen content (per cent.) of the plant at this stage varies from variety to variety, early duration varieties showing a higher nitrogen content than the later duration ones.

3. Absorption process of nitrogen by all varieties shows that, during growth phase, rate of nitrogen absorption attains a maximum value, once at the maximum tiller number stage, which is common for all the varieties and second at ear development stage. In early duration varieties, these two stages almost follow each other, making the process of nitrogen uptake by these varieties continuous right up to the flowering stage. In the medium and late duration varieties, during the period of lag between maximum tiller number stage and ear development stage, the rate of nitrogen uptake slows down considerably and becomes vigorous again with ear development. These two peak periods of nitrogen uptake by different duration varieties correspond

to two peak periods of *partial efficiency*, described by Tanaka *et al.* (1959), when nitrogen absorbed by the plant is efficiently utilized for production of grain.

In view of these results, it is considered that split application of fertilizer, one dose at planting and second at ear-initiation stage, will be beneficial for increasing yield, particularly of the medium and long duration varieties.

The authors are indebted to Mr. R. L. M. Ghose, Director, Central Rice Research Institute, Cuttack, for his suggestions in improving the text of the paper.

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STUDIES ON INDIAN MYMARIDÆ—PART I

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(Division of Entomology, Indian Agricultural Research Institute, New Delhi)

Received December 1, 1958

The family Mymaridæ comprises some of the smallest insects that are well distributed. The members of this small but interesting group are parasitic on the eggs of other insects, particularly that of Hemiptera. Several species of this group have been successfully utilized in the biological control of certain serious pests. A good deal of work has been done on the taxonomy of Mymaridæ by various workers in other parts of the world. In India, the work done on Mymaridæ almost amounts to nil. Mani (1938) included only 6 species in his catalogue of Indian Chalcidoidea. Later the same author (1942) described 3 species new to science. However, since then, not a single species has been added to our fauna, though we have several genera and a good number of species belonging to Mymaridæ. The authors have been collecting insects for sometime past and their taxonomic portion is being studied. It is intended to publish a series of papers on Indian Mymaridæ. The types have been deposited in the National Pusa Collection, Indian Agricultural Research Institute, New Delhi.

Lymænon narayani sp. nov.

Female

Length.—0·975 mm. (inclusive of the ovipositor).

Colour.—General colour yellow with brown spots all over.

Head.—Slightly more than one and one-third times as wide as long. Vertex prominent like all other species. Eyes red, elongate. Genal space slightly more than half the length of eyes. Ocelli red and arranged in a low arc. Interocular space thrice the interocellar and four times the ocellocular; the latter thrice the frontocellar. Antennæ brown, situated slightly below the middle of the head; sockets thrice their own diameter apart. The relative lengths of the antennal segments from scape onwards as follows:

20, 8, 4, 3, 3·5 4, 7, 7, 7, 7, 23.

Club solid, about three and one-thirds its own breadth, with five linear sensillæ. F_4–F_8 with one or more sensillæ in each [Fig, 3 (1)].

Thorax.—Very faintly reticulate; parapsidal furrows weakly defined, upper half of the scutellum brown, lower yellow. Scutum almost equal to

that of scutellum; metathorax narrow, propodeum brown, with a spiracular sulci at the sides.

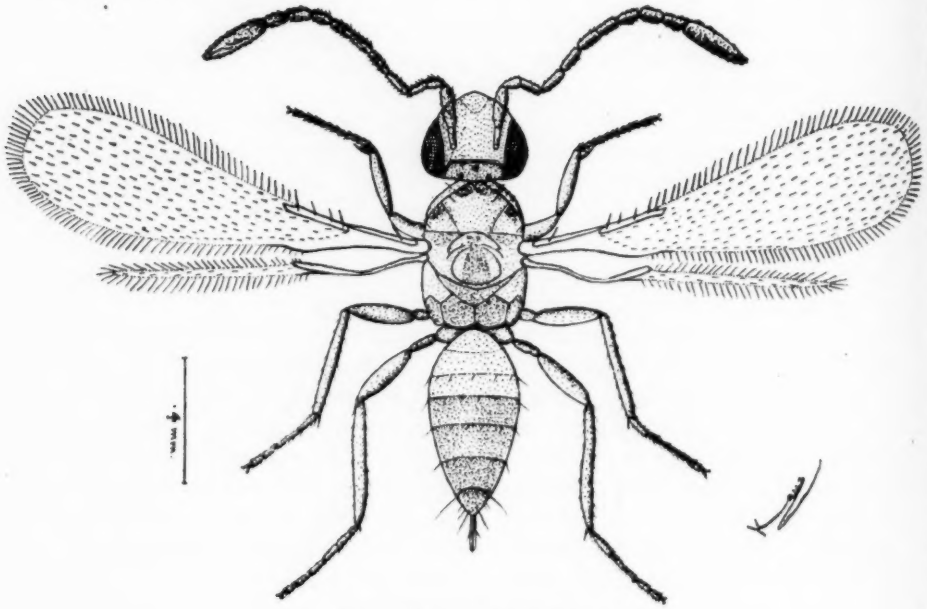

FIG. 1. *Lymænon shasthryi* sp. nov.

Abdomen.—Conicovate, faintly reticulate, upper half of the dorsum yellow, lower brown. A row of spines present in each segment. The first row consists of only two. Ovipositor slightly exserted and starts from behind the tip of the abdomen.

Legs.—Slender, coxæ and femurs yellow; tibia and tarsi somewhat brownish in shade. Hind tibia equal to the combined length of its femur and trochanter as well as equal to its tarsus.

Wings.—Forewing three and one-third times as long as broad, a little longer than the hind wing. Submarginal one and a half times the length of the marginal. Surface hair numerous, start from just below the proximal end of the marginal vein and are evenly distributed. Marginal cilia short; the longest being a little less than one-third the greatest wing width [Fig. 2 (1)].

Described from two females.

Holo type.—On slide.

Host.—Unknown (Sweeping lucerne fields).

Col.—R. B. Kaur; May 15, 1957.

Locality.—Delhi.

Lymænon indicus sp. nov.

Female

Length.—About 1·17 mm.

Colour.—Head, thorax, coxæ of the forelegs, tibia of the hind legs, distal eight segments of the antennæ brown; rest of the antenna and legs

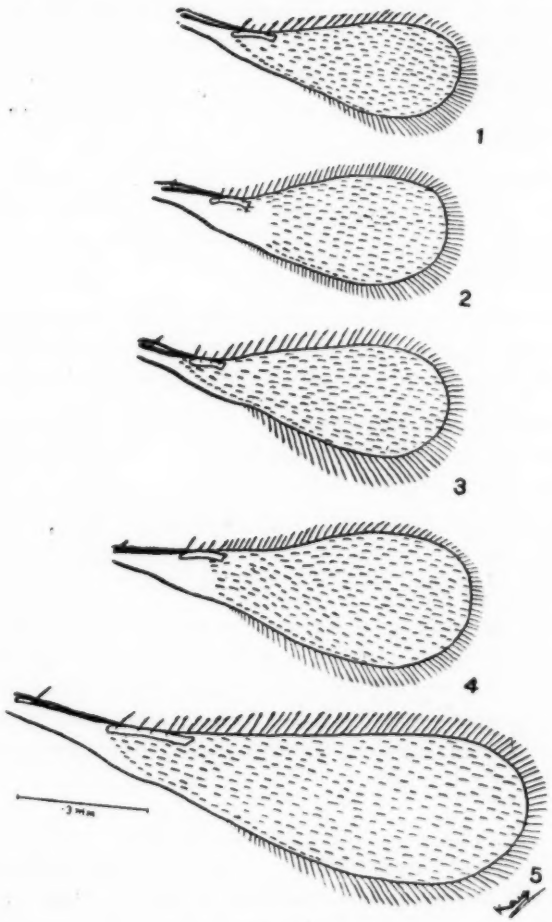

FIG. 2

FIGS. 2 and 3. Forewings and antennæ of *Lymænon* spp. (1) *L. narayani;* (2) *L. sahadevani;* (3) *L. ramakrishnai;* (4) *L. indicus;* (5) *L. shasthryi.*

yellow. Abdomen gives a banded appearance. Dorsally, the upper half of each segment is lemon in shade whereas the rest of its is brown. Ventrally it is yellow. The proximal half of the abdomen lighter in shade as compared to the distal half.

Head.—Slightly wider than long and a little wider than thorax. Vertex broad as usual and very faintly reticulated. Eyes deep red ocelli very prominent and form an obtuse angled triangle. Interocular space about twice the interocellar; frontocellar space slightly less than ocellocular and almost half the interocellar. There are six prominent spines on the head; two between the ocelli, two above it; and two just below the eyes. Antennæ slightly hairy. Scape compressed laterally and from its lateral aspect thrice as long as thick. Relative lengths of the antennal segments from scape onwards as follows:

22, 8, 5, 5, 8, 7, 8, 7, 7, 6, 28.

Club solid, oblong, a little wider than the preceding segments, with four linear sensillæ. Each of the funicular segment except the first two with one such sensilla [Fig. 3 (4)].

Thorax.—Smooth, beset with a few spines on the pro- and meso-thoracic area as in all other species. Parapsidal furrows complete and weakly marked. Scutum almost equal to that of scutellum. Metathorax narrow.

Abdomen.—Subsessile, concovate. A row of seven spines present in each segment except the first where they are a few. Ovipositor slightly exserted and starts from behind the tip of the abdomen.

Legs.—Moderately developed. Hind tibia about one and a half times longer than its femora but almost equal to the combined length of femur *plus* trochanter; hind tarsus equal to its tibia.

Wings.—Forewings about two and three-fourth times longer than broad, a little longer than the hind wing, submarginal vein about one and a half times the length of marginal. Marginal cilia short, the longest being one-sixth the greatest wing width. Surface hair start from beyond the middle of the marginal and are evenly distributed over the rest of the surface [Fig. 2 (4)].

Male

Exactly similar to female except in antennæ and wing ratio. Scape and pedicel almost as long as wide, the former slightly longer than the latter. The rest of the segments are almost equal and with sensory lists. Forewings about thrice as long as wide.

Described from a single female and a single male.

Holo and Allo types on slides.

Coll.—R. B. Kaur; May 17, 1957.

Host.—Unknown (Sweeping lucerne).

Locality.—New Delhi.

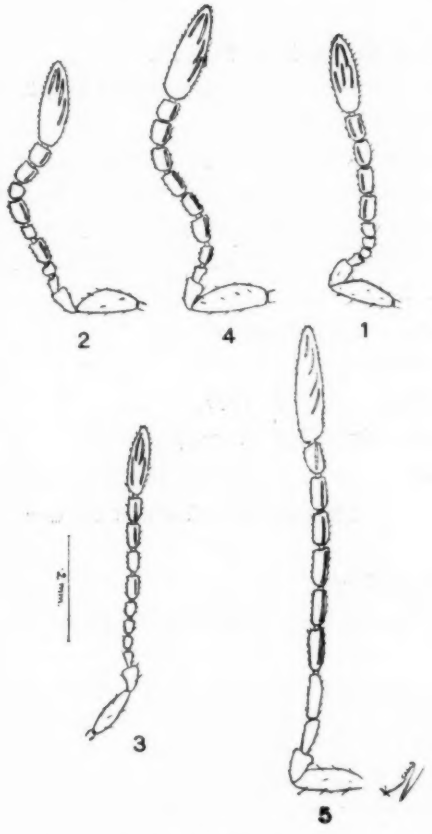

FIG. 3

FIGS 2. and 3. Forewings and antennæ of *Lymænon* spp. (1) *L. narayani*; (2) *L. sahadevani*; (3) *L. ramakrishnai*; (4) *L. indicus*; (5) *L. shasthryi*.

Lymænon sahadevani sp. nov.

This species comes very close to *L. indicus* except for some of the characters;

Female

Length.—1·04 mm.

Colour.—Similar to *L. indicus* except the abdomen and the pre-tarsi, which are darker in shade.

Antennæ scape and pedicel yellow, rest of the antennæ brown. It differs from *L. indicus* in the relative lengths of its segments which run as follows:

18, 8, 4, 3, 7, 4, 6·5, 4·5, 6, 5·5, 24.

Linear sensillæ are present in the club and F 3, 5, 7 and 8 [Fig. 3 (2)].

Thorax.—Similar to *L. indicus.*

Abdomen.—Brown both dorsally as well as ventrally. The upper one-third of each segment is lemon and the rest of it is perfect brown.

Wings.—Forewings three times as long as broad. A little shorter in length as well as in breadth than *L. indicus.* Surface hair start from beyond the marginal [Fig. 2 (2)].

Described from three females.

Holotype.—on slide.

Coll.—R. B. Kaur; July 5, 1957.

Host.—Unknown (Sweeping lucerne).

Locality.—Delhi.

Lymænon ramakrishnai sp. nov.

Female

Length.—About 0·88 mm.

Colour.—Body brown with dark sutures, joints of the legs lighter in shade.

Head.—About one and one-third times wider than long. Eyes red, bare, elongate, slightly less than one and a half times the length of cheeks. Interocular space about twice the interocellar. The latter about twice the ocellocular. Antennæ brown, slightly pubescent, situated on the middle of the face close to the eye borders; sockets twice their own diameter apart. Scape cylindrical and from its lateral aspect four times longer than broad. The relative lengths of the antennal segments as below:

16, 7, 4·5, 3·5, 4, 4, 7, 6, 7, 6·5, 20.

Club solid, conical, a little more than three and a half times longer than wide with four prominent sensillæ; Funicular segments 5, 7 and 9 also with one sensilla in each. [Fig. 3 (3)].

Thorax.—Completely brown, slightly convex; parapsidal furrows complete and well impressed. Scutum a little smaller in length to that of scutellum. Spiracular sulci prominent.

Abdomen.—Similar to that of *L. sahadevani.*

Legs.—Slender, hind tibia equal to the combined length of its femur *plus* trochanter and slightly less than its tarsus. Foretibia almost equal to its femur.

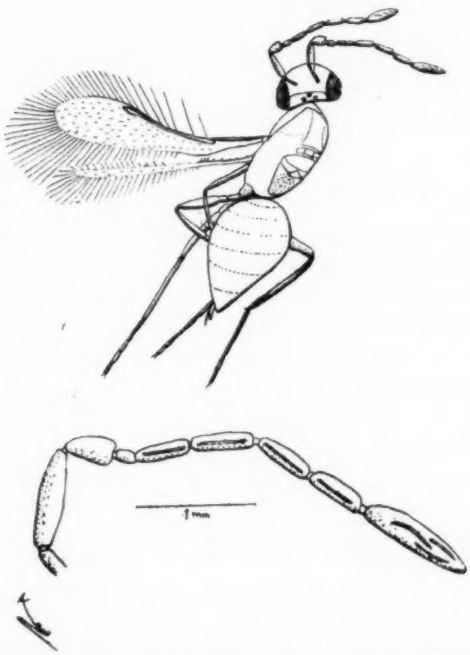

FIG. 4. *Neurotes enockii* sp. nov. showing also an enlarged antenna.

Wings.—Forewing about three times its own breadth and about one-thirds the length of hind wing. Submarginal weak and a little less than twice the length of the marginal, which is slightly thicker as compared to the marginal vein of *L. narayani.* Marginal fringe normal, the longest cilia a little less than one-half the greatest wing width. Surface hair start from just before the marginal and are equally distributed over the rest of the surface. [Fig. 2 (3)].

Male

Length.—0·87 mm. Exactly similar to female except for the antennæ. Scape a little longer than wide; pedicel almost as long as wide and slightly

less than the length of the scape. Eyes orange yellow in colour (probably due to long preservation).

Described from four females.

Holo and Allo types, on slides.

Coll.—R. B. Kaur; May 5, 1957.

Locality.—Delhi.

Host.—Unknown (Sweeping lucerne fields).

Lymænon shasthryi sp. nov. (Fig. 1)

Female

Length.—1·2–1·35 mm. This is the largest of the species collected so far.

Colour.—Body yellow with brown spots.

Head.—Slightly wider than thorax and a little broader than long. Brown with eyes and vertex dark in shade. Eyes longer than broad and bare. Genal space less than the length of the eyes. Interocular space a little more than three times the interocellar, the latter almost twice the frontocellar as well as ocellocular. Towards the proximad, there is a pair of stout spines on each side of the vertex. Antennæ long, slightly hairy and placed close to the mid inner border of the eyes. First three segments lighter in shade whereas the rest of the antenna dark brown. Scape cylindrical. Relative lengths of the segments from scape onwards as follows:

20, 9, 10, 13, 12, 11, 11, 10, 10, 9, 34.

Funicular segments slender and almost of equal thickness. Club solid and just four and a half times longer than wide [Fig. 3 (5)].

Thorax.—Elongate, moderately convex and with a faint median groove. Prothorax light brown and with a pair of spines on each side of it. Pleuron brown. Parapsidal furrows distinct, axillæ brown and separated. Scutum with a big brown spot in its anterior region. Scutellum also with a big oval brown spot in its middle and slightly less than scutum in its median length. Metathorax slightly curved into the region of the propodeum. Propodeum brown with four carinæ, the two middle ones are hexagonal in shape and the side ones, which are comparatively smaller, are subrectangular.

Abdomen.—Elongate, broad in the centre, gradually and uniformly tapering to a point distally. First three tergites are brownish yellow whereas the last four are dark brown in colour except the anal extremity which again is lighter in shade. A row of spines is present on each segment except the

first one. A pair of spines present on the sheeth of ovipositor, one on each half. Ovipositor exserted to about one-sixth the length of abdomen.

Legs.—Brownish yellow, coxæ comparatively darker in shade. Hind femora slightly more than half the length of its tibia, the latter one and one-fifth times longer than its tarsus.

Wings.—Forewing varies from being three and one-third times longer than broad and one and one-third times longer than the hind wing. Submarginal a little longer than the marginal. Marginal cilia short but uniformly distributed, the longest being a little more than one-fourth the greatest wing width. Surface hair numerous and uniformly distributed all over. [Fig. 2 (5)].

Male

Length varies from ·9 to 1·17 mm.

Coloration similar to that of female. Antenna longer than the body and measures to about 1·4 mm. in length. Pedicel half the length of the first funicular segment; except these two the rest of the antennal segments are almost equal. Segments beyond the pedicel with linear parallel sensillæ. Club solid and oblong.

Thorax.—Like that of female but no carinæ are seen on the propodeum.

Abdomen.—Oblong and almost equal to the length of the thorax. Arrangement of spines as in female.

Wings.—Similar to that of female.

Legs.—Long and slender. Hind tibia equal in length to that of its tarsus.

Described from six females and two males.

Holo and Allotypes on slides.

Coll.—B. R. Subba Rao.

Locality.—Mandya (Mysore State).

Host.—Tettigonella spectra (Dist.) (Fulgoridæ: Hemiptera).

It gives us great pleasure to name the species after Mr. Shivashankar Shasthry, who first studied the biology of the host and recorded the parasite.

Neurotes enocki sp. nov. (Fig. 4)

Female

Length.—0·676 mm.

Colour.—Brownish yellow.

Head.—Viewed from above subcomplanate, almost one and a half times broader than long and about as broad as thorax. Eyes bare, red (dark in old mounts) and elongate. Genal space about half the eye length. Ocelli red and lie in a low arc. Interocular space three times the interocellar, the latter slightly over the ocellocular, vertex brown. Antenna eight-jointed, brownish yellow and uniformly coloured, non-hairy shorter than the length of the body. The relative lengths of the antennal segments as below:

12, 6, 2·5, 7, 8, 8, 7, 28.

Scape cylindrical; pedicel one and a half times longer than broad; F_1 slightly longer than broad; the following four about thrice their own breadth. Club solid, rounded at the tip where there are a few very minute hair; four times longer than broad. Sensory ridges present on the distal four funicular segments as well as on the club but the exact number cannot be made out as they are very faint.

Thorax.—Oblong, faintly striated longitudinally. Anterior tip of the mesothorax brown; a brown spot in the region of parapsids. Parapsidal furrows not deeply impressed. Just at the base of scutum, in front of the anterior border of the scutellum, there is a belt of three rectangular carinæ with the largest in the middle. Scutellum smooth and smaller than scutum. Metathorax with one median diamond-shaped carina. Propodeum somewhat brownish and feebly sculptured; from the lower tip of the metathoracic carina 12–16 faint longitudinal lines radiate towards the base of the propodeum (shown in Fig. 4).

Abdomen.—Subpetiolate, smooth and conical; slightly longer than thorax. First tergite the smallest and then the size increases gradually up to sixth, which is the largest; seventh is a small triangular piece. Ovipositor slightly exserted and starts from behind the tip of the abdomen.

Legs.—Lemon yellow, long and slender; coxæ moderately developed; trochanters two-jointed. Foretibia comparatively thicker and shorter. Hind tibia slender and slightly more than one and a half times the length of its femur. Tarsi pentamerus, segments equal, hind tarsus of the length of its own tibia, foretarsus longer than its tibia and almost equal to its femur.

Wings.—Forewings long and narrow with the costal edge a little convex; four and a half times longer than wide. Marginal cilia long, dense and cover the whole wing margin except some basal portion; the longest is a little over the greatest wing width. Surface hair start from just below the proximal end of the marginal. Submarginal thin and short. Marginal very long, reaching to beyond the middle of the wing, the tip slightly

enlarged. Post-marginal and stigmal absent. Hind wings narrow and linear; a little smaller than the forewings densely ciliated but cilia not longer than those of the forewing.

Described from a single female on slide.

Coll.—R. B. Kaur; June 20, 1957.

Locality.—I.A.R.I., New Delhi.

Host.—Unknown (Sweeping lucerne and mulberry).

Key to Females of Indian Species of Lymænon *Haliday*

1.	Thorax evenly brown	2
	Thorax yellow with brown patches	4
2.	Second antennal segment almost equal to that of fifth	3
	Second antennal segment appreciably larger than the fifth; surface hair of the forewing start from just before the marginal vein	*L. ramakrishnai* sp. nov.
3.	F_5 to F_9 of antenna almost equal, and provided with sensory lists; surface hair of forewing start from beyond the middle of the marginal vein	*L. indicus* sp. nov.
	F_5 to F_9 of antenna unequal; F_6 and F_8 without sensory lists; surface hair of forewing start from beyond the marginal vein	*L. sahadevani* sp. nov.
4.	Funicular segments almost equal in lengths	5
	Funicular segments unequal in lengths: First four funicular segments appreciably smaller than the last four; surface hair of the forewing start from just below the marginal vein	*L. narayani* sp. nov.
5.	Funicular segments almost equal in width; surface hair of forewing start from just below the marginal vein	*L. shasthryi* sp. nov.
	Last two funicular segments appreciably wider than the rest.	*L. longicrus* (Kieffer).

Key to the Species of Neurotes *Enock.*

Females

1. Head and thorax dark brown or smoky brown; wing surface not hyaline — 2

   Head and thorax brownish yellow; wing surface hyaline; hind tarsus over one and a half times as long as its femora; first funicular segment one-fifth of scape. — *N. enocki* sp. nov.

2. Head and thorax dark brown; surface hair most numerous at the base and margins of the wing leaving an almost clear area in the centre; second funicular segment the longest. — *N. iridescens* Enock

   Head and thorax smoky brown; pilosity of the wing infumate; hind tarsus as long as its femora; first funicular segment one-third of scape. — *N. flaviventris* Ryland

Note.—It is rather difficult to draw a key to species without well differentiated specific characters of the described species. In the case of *N. iridescens* Enock, description of the female is not much detailed and the ratio of various components of the legs have been neglected altogether; whereas, in the case of *N. flaviventris* Ryland, legs have been fully described but the ratio of various components of female antenna is not given because of the breakage of antenna, above second funicular joint. In the absence of characters made out by examining the type specimens and with the available original description a key, more or less practicable, has been drawn.

ACKNOWLEDGMENTS

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* The original not seen.

CHROMOSOME NUMBERS IN INDIAN MEDICINAL PLANTS—III

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(Communicated by Dr. E. K. Janaki Ammal, F.A.SC.)

The paper deals with the chromosome numbers of 13 species of Indian medicinal plants from the collection of medicinal plants grown for genetical studies in the Central Botanical Laboratory. For pollen mother cell studies, flowers were fixed in acetic alcohol and stained in acetocarmine. Somatic squashes of a few species were made after prefixing with 8-oxyquinoline and staining in acetic orcein, following the schedule by Tjio and Levan (1950). Materials were also fixed in chrome-acetic-formalin sectioned at 12 μ and stained in crystal violet. Camera lucida drawings were made and the appropriate magnification is given at the end of each drawing.

This list includes 5 genera, namely, *Bacopia*, *Holarrhena*, *Cicca*, *Sonneratia* and *Evolvulus*, which have been worked out for the first time. Chromosome counts of 10 species have been determined for the first time and in 3 other species marked * the present observations differ from the previous reports by other workers.

Of the new genera, *Bacopia monnieri*, belonging to the family Scrophulariaceæ, is the reputed Brahmi plant in the Indian pharmacology. It is found growing in wet marshy places and was collected by Dr. E. K. Janaki Ammal from Dehra Dun, Madras and Kerala. The Dehra Dun form had slightly larger leaves than the Madras form. The oil extracted from the stems and leaves of this plant contains the alkaloid brahmine and is used in the treatment of Asthma and insanity besides as a nerve tonic. All the plants collected were found to have a diploid number of $2n = 64$ chromosomes.

Holarrhena antidysenterica, belonging to the family of Apocynaceæ, is a small tree found all over India. The bark and the seeds of this tree are of great medicinal value and many valuable alkaloids have been isolated from this plant which are used in the treatment of fever, dysentery, diarrhœa and intestinal complaints. Materials for the present study were collected from Dehra Dun and the haploid number was found to be $n = 11$ which is very common in the family Apocynaceæ.

Cicca acida, an Euphorbiaceous tree, is commonly cultivated in South India for the fruit which is pickled or made into preserves. Till very recently the plant was known as *Phyllanthus distichus* Muell. Arg. The parts valued medicinally are the fruits, leaves, roots and seeds. The fruit is acid and astringent; the seed is cathartic and the root an active purgative. The diploid number of this plant has been found to be $2n = 26$ with a basic number of $x = 13$ and is, therefore, a diploid like *P. niruri* (Sundara Raghavan, 1957).

Sonneratia apetala belonging to the family Sonneratiaceæ, is a mangrove plant and was collected by Dr. R. P. Patil, Botanist, from Salsette Island in Bombay State. The sub-acid fruits are edible and the plant is valued for its tannin. Meiosis is quite regular and the haploid number of this species has been found to be $n = 9$.

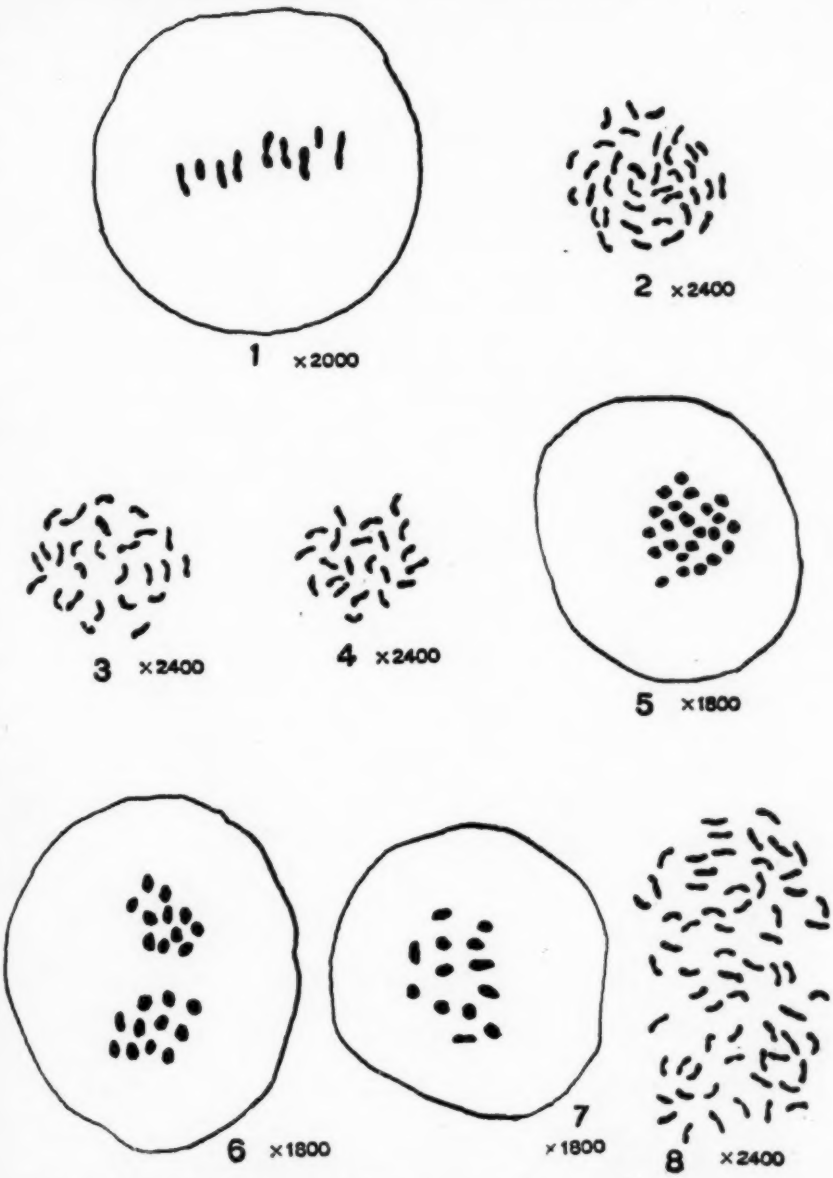
Evolvulus alsinoides is the famous Vishnugranthi of Ayurvedic medicine and is a common weed found throughout India. The leaves of this plant when made into cigarettes and smoked are said to have soothing and curative effect for people suffering from Asthma and chronic bronchitis. This plant yields a bitter tonic and is used in the treatment of dysentery. It has a diploid number of $2n = 26$ with a basic number of $x = 13$ which is quite a new number for the family Convolvulaceæ.

Celastrus paniculatus of Celastraceæ, collected from Dehra Dun, has a haploid number of $n = 23$ which is the same as the numbers reported by Bowden (1940, 1945) for a few other species examined by him. The bark and seeds of this plant are used in rheumatism, leprosy and paralysis.

Curcuma angustifolia of the family Zingiberaceæ is found growing wild but is also cultivated in the South for its edible rhizomes. The rhizomes contain starch which is used as a substitute for arrowroot powder normally obtained from *Maranta arundinacea*. The genus *Curcuma* has two basic numbers of $x = 16$ and 21. Somatic counts of this plant reveal $2n = 64$ chromosomes which is also shared by a few other species of *Curcuma* with a basic number $x = 16$ as *C. longa*, *C. zeodaria* and *C. petiolota* (Venkatasubban, 1946).

Pandanus tectorius Roxb., belonging to the family Pandanaceæ, is valued mainly for its aromatic oil distilled from the bracts and is a common plant in South India. The diploid number of this plant was found to be $2n = 60$.

Putranjiva roxburghii (Euphorbiaceæ) is very much valued for its leaves, fruits and stones that are used medicinally in colds and fevers. The plant examined in the present case was collected from Dehra Dun and its somatic number was found to be $2n = 40$. Perry (1943) has, however, reported $2n = 14$ for the material examined by him.



TEXT-FIGS. 1-8. Fig. 1. *Sonneratia cpetcla* (M_1; $n = 9$), ×2,CC0. Fig. 2. Scmatic metaphase of *Grewia tenax* showirg $2n = 36$ clrcmoscmes, ×2,4C0. Fig. 3. Scmatic metaplase of *Cicca acida* showing $2n = 26$ chrcmoscmes, ×2.4C0. Fig. 4. Scmatic metaplase cf *Psoralea corylifolia* showirg $2n = 22$ clrcmoscmes, × 2,4C0. Fig. 5. *Celcstrus paniculatus* (M_1; n=23) × 1,800, Fig. 6. *Holarrhena antidysenterica* (M_1; $n = 11$), × 1,8C0. Fig. 7. *Evolvulus alsinoides* (M_1; n = 13), × 1.8C0. Fig. 8. Scmatic metaplase cf *Eccepia monnieri* showing $2n = 64$ chrcmoscmes, × 2,4C0.

Grewia tenax, a Tiliaceous plant, is found growing in the dry arid tract of Punjab, Rajputana, Western India and South India. Recently the Russians have ascribed to this plant curative properties for tuberculosis as a result of which this plant has attained much publicity. The red berries of this plant are eaten in parts of Punjab. The chromosome number of this plant was found to be $2n = 36$ with a basic number of $x = 9$.

Psoralea corylifolia, a Papilionaceous plant, is coming into prominence because of the remarkable curative properties of this plant in the treatment of skin diseases as leucoderma and leprosy. It is mainly the seeds that are very much valued in the treatment of the above diseases. The seeds yield an oleo-resin and an essential oil on extraction which contain the active principles psoralen and isopsoralen (Chopra *et al.*, 1956). In a recent paper, Agarwal and Tayyab (1958) have reported $n = 10$ for the species examined by them. The present cytological observations were carried out on plants grown from seeds obtained from Calcutta. Somatic squashes, however, reveal well-spread metaphase plates with $2n = 22$ chromosomes. Turner (1956) has reported $n = 11$ for four species of *Psoralea* examined by him namely in *P. cyphocalyx*, *P. digitata* var. *parviflorus*, *P. hypogæa* and *P. rhombifolia*. The present observations agree with the findings of Turner as regards to the basic number of the genus.

Strychnos nuxvomica, belonging to the family Loganiaceæ, contains the alkaloids strychnine and brucine which can be extracted from the leaves, bark, wood and roots. The plant examined was grown from the seeds obtained from a tree growing in the National Botanical Gardens, Lucknow, and it revealed $2n = 44$ chromosomes in somatic counts. The previous number reported for the species is $2n = 24$ (Janaki Ammal, 1945) and it is probable that the present plant is an aneuploid derivative from the tetraploid $(4x - 4)$.

The following is the list of chromosome numbers determined:—

Family and Name	Vernacular names	Chromosome number
Sonneratiaceæ		
Sonneratia apetala Ham. ..	Hindi—Keowra	$n = 9$ ($2n = 18$)
Tiliaceæ		
Grewia tenax (Forsk) Fiori. syn. *G. Populifolia* Vahl.	Telugu—Kaladi	$2n = 36$

Family and name	Vernacular names	Chromosome number
Euphorbiaceæ		
Cicca acida (L.) Merrill ..	Hindi—Harfarauri Tamil—Arnalli	$2n = 26$
**Putranjiva roxburghii* Wall.	Hindi—Putranjiva	$2n = 40$
Papilionaceæ		
**Psoralea corylifolia* L. ..	Hindi—Babchi Tamil—Karpo karishi	$n = 11$ ($2n = 22$)
Celastraceæ		
Celastrus paniculatus Willd. ..	Hindi—Malkangni Tamil—Valulurai	$n = 23$ ($2n = 46$)
Mrysinaceæ		
Ardisia solanacea (Poir) Roxb. ..	Tamil—Manipudbam	$2n = ca\ 46$
Loganiaceæ		
**Strychnos nuxvomica* L. ..	Hindi—Kuchla Tamil—Etti	$2n = 44$
Apocynaceæ		
Holarrhena antidysenterica Wall. ..	Hindi—Kurchi Tamil—Kudasappalai	$n = 11$ ($2n = 22$)
Convolvulaceæ		
Evolvulus alsinoides L. ..	Hindi—Sankhapushpam Tamil—Vishnukranthi	$2n = 26$
Scrophulariaceæ		
Bacopia monnieri (L.) Pennel ..	Hindi—Brahmi Tamil—Nirbrahmi	$2n = 64$
Zingiberaceæ		
Curcuma angustifolia Roxb. ..	Hindi—Tikhur Tamil—Kua	$2n = 64$
Pandanaceæ		
Pandanus tectorius Soland ex- Parkinson	Hindi—Keora Tamil—Thazhambu	$2n = 60$

SUMMARY

The paper deals with the chromosome numbers of 13 plants of medicinal value. The chromosome counts of 10 species have been reported for the first time.

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STUDIES ON THE SUCCESSION OF MARINE ALGÆ ON A FRESH SUBSTRATUM IN PALK BAY

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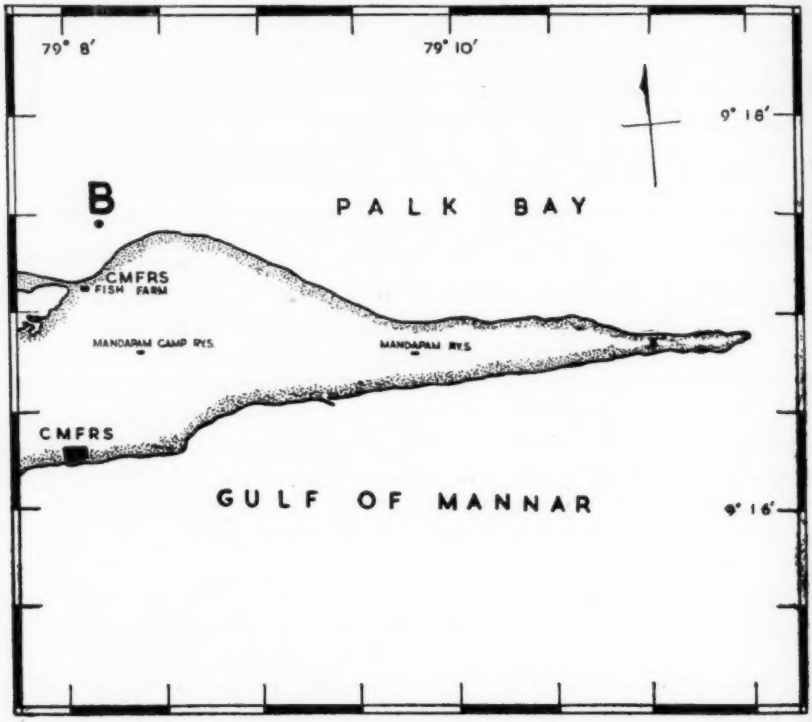
Despite the numerous investigations on the ecology of marine plants, the subject has not advanced as much as the ecology of terrestrial plants, because, unlike the study of land vegetation, field experiments are more difficult in marine environments. In this connection, the studies of Gail (1918, 1922), of Hatton (1938) and of Rees (1940) indicate the possibility and essentiality of such experimental studies.

Contributions on the ecology of Indian marine algæ are limited to a few papers. Iyengar (1927) published a brief account on the algæ of Krusadai Island. Boergesen (1930, 1931, 1932 *a*, 1932 *b*, 1933 *a*, 1933 *b*, 1934, 1937 *a*, 1937 *b* and 1938) gave some notes on the ecology of the more important species in his papers on the taxonomy of the marine algæ of the Sub-Continent, while, Srinivasan (1946) and Krishnamurthy (1954) dealt with certain aspects of marine algal ecology with reference to Mahabalipuram and the estuary of the Adyar River respectively. From the neighbourhood of India, we have the studies of Svedelius (1906 *a* and 1906 *b*) on the vegetation of a coral reef of Ceylon and on the ecology and systematics of Ceylon species of *Caulerpa* as well as the publications by Anand (1940 and 1943) which include ecological accounts on the algal flora of the Karachi coast.

The present work, carried out over a period of 18 months, is on the growth and succession of marine algæ on a fresh substratum in theinter -tidal zone in Palk Bay. The place chosen for the study is at a distance of 76·2 metres from the mean shore line. The mean water level at low tide is 1·37 metres; while at high tide it is 1·82 metres. The substratum is mostly sandy; about 10 metres from the experimental spot toward the shore, the substratum has a dominance of corals, mostly dead ones; toward the left of the spot the shore is sandy clay. But for a small channel about 400 metres from the mean shore line, the Bay experimental area is more or less uniformly shallow (Text-Fig. 1).

A concrete boulder 1·22 metre square, which forms the fresh substratum for the experimental studies, is placed in position at the experimental area by means of a wooden tripod erected at the spot. The top surface of the boulder is paved with corals. The boulder is lifted up periodically for observations and kept back in position with the help of chain blocks (Plate IV, Fig. 1). The experiment was started in the month of February, when the Bay was more or less calm with only occasional turbulence.

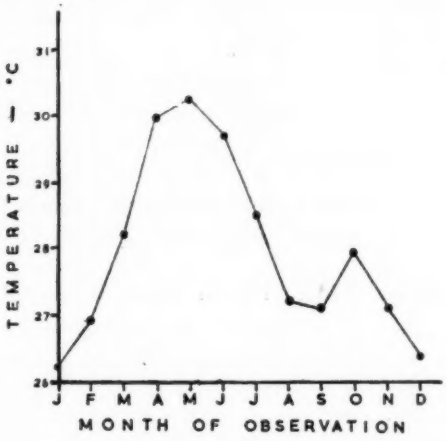


TEXT-FIG. 1. Map of Palk Bay showing the experimental site B.

GENERAL FEATURES OF THE BAY

In Palk Bay, the sea is perfectly calm during the months of March to August, it becomes rough by the middle of September, continues to be rough throughout the North-East Monsoon period, and becomes turbulent during the months of December and January. During February, the sea is calm or turbulent depending on the changing winds. Some of the chemical data referring to Palk Bay have been taken from Jayaraman (1954), and tempera-

ture and total nitrogen values were obtained by the writer (Text-Figs. 2 and 3). Oxygen content values are steady all year round and never reach the saturation point at any period. Similarly, phosphate values also do not show any monthly or seasonal fluctuations of marked intensity. Salinity data show two distinct phases, a rise from July to October and a fall from November to January. The period from March to June may be considered as one of moderate salinity. Silicate values indicate a distinct rise from September to November remaining more or less moderate from January to August. Regarding total nitrogen, there is a rhythmic rise and fall; the peak months being February-March, June-July and September-October. Temperature data show that the range is within about 4° C., the warmest period being from April to June.



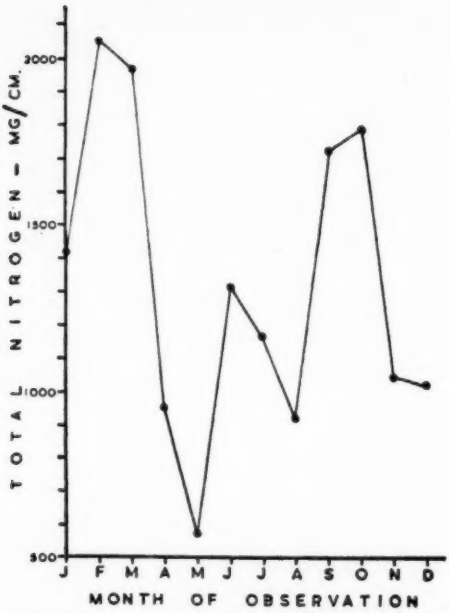
TEXT-FIG. 2. Monthly variations in the surface temperature of sea-water in Palk Bay.

TYPES OF SUBSTRATA COLONIZED

1. *A fresh coral surface.*—The upper surface of the concrete boulder (1·5 sq.M.) was paved with coral stones, previously dried and cleaned.

2. *Fresh concrete surfaces.*—The four vertical sides of the boulder (each side 1·5 sq.M.) were of unpolished concrete. The sides were marked as faces 1, 2, 3 and 4; face 1 facing the shore (South), faces 2 and 4 at right angles to the shore line (West and East respectively) and face 3 facing the open Bay (North).

3. *Wooden surfaces.*—The horizontally placed palmyra beams used for keeping the leverage-tripod in position around the boulder.



TEXT-FIG. 3. Monthly variations in the total organic nitrogen content of sea-water in Palk Bay.

ALGÆ CONCERNED IN THE COLONIZATION

(*A*) *Chlorophyta*

1. *Ulva reticulata* Forsskål
2. *Cladophora* sp.
3. *Chætomorpha media* (Agardh) Kützing
4. *Caulerpa peltata* (Turner) Lamouroux
5. *Caulerpa racemosa* (Forsskål) J. Agardh
6. *Caulerpa taxifolia* (Vahl) C. Agardh
7. *Acetabularia mœbii* Solms-Laubach
8. *Neomeris annulata* Dickie
9. *Bœrgesenia forbesii* Harvey

(*B*) *Chrysophyta*

10. *Licmophora abbreviata* Agardh
11. *Climacosphœnia elongata* Bailey
12. *Cocconeis* sp.
13. *Navicula* spp.
14. *Nitzschia closterium* (Ehrenberg) W. Smith

(*C*) *Phæophyta*

15. *Sphacelaria tribuloides* Meneghini
16. *Dictyota bartayresiana* Lamouroux
17. *Dictyota maxima* Zanardini
18. *Padina gymnospora* (Kützing) Vickers
19. *Sargassum myriocystum* J. Agardh

(*D*) *Rhodophyta*

20. *Liagora farinosa* Lamouroux
21. *Gelidium micropterum* Kützing
22. *Amphiroa fragilissima* (Linnaeus) Lamouroux
23. *Jania rubens* (Linnaeus) Lamouroux
24. *Lithophyllum* sp.
25. *Hypnea esperi* Bory
26. *Hypnea valentiæ* (Turner) Montagne
27. *Gracilaria lichenoides* (Linnaeus) Harvey
28. *Champia globulifera* Boergesen
29. *Champia parvula* (Agradh) Harvey
30. *Chondrococcus hornemanni* (Mertens) Schmitz
31. *Ceramium transversale* Collins *et* Hervey
32. *Spyridia fusiformis* Boergesen
33. *Roschera glomerulata* (Agardh) Weber van Bosse
34. *Acanthophora spicifera* (Vahl) Boergesen
35. *Laurencia obtusa* (Hudson) Lamouroux
36. *Laurencia papillosa* (Forsskål) Greville
37. *Leveillea jungermannioides* (Martens *et* Hering) Harvey

(*E*) *Cyanophyta*

38. *Lyngbya majuscula* Harvey
39. *Symploca hydnoides* Kützing

Phases of Colonization

(*a*) *Pioneer Communities*

It is well known that the nature of the pioneer community on a fresh substratum depends on the season of the year. In the present studies, the first colonizers on the substratum were observed after a period of 20 days from the date of starting the experiment, when, on the coral surface were seen 14 plants of *Chætomorpha media*, about 1·5 cm., in height, along with a fine uniform spread of *Padina gymnospora* attaining a height of 5–10 mm. There were a few *Lyngbya majuscula* growing in between the *Chætomorpha*

media plants. On the concrete vertical faces, toward the upper regions, *Padina gymnospora* sporelings of the same growth size as on the coral surface were seen, except on face 3 where there was poor colonization by it. There was no algal growth on the palmyra beams.

(*b*) *Succession*

(*i*) *Coral surface.*—On the coral surface, it was observed that the spread of *Padina gymnospora* was very fast and by the end of March, they almost replaced all the *Chætomorpha media* which by then had attained a height of 5 cm. *Padina gymnospora* was seen all year round, but it was more dominant and luxuriant during the period February to April (Plate IV, Figs. 2 and 3). By the beginning of June, most of the plants were either eaten off by grazing animals or torn off their holdfasts. A second crop of *Padina gymnospora* was seen coming to prominence by the end of November, attaining a moderate sized population in December. This second crop was only of a short duration; by the middle of January most of the plants were washed off their hold. The cycle was again repeated by the end of February.

Early in March a fine growth of *Acetabularia mœbii* appeared on the coral surface, but before the plants could attain full growth they were eaten by nudibranchs which appeared during this season. A fresh crop again appeared by the middle of April, which was also eaten off by nudibranchs. By the end of May, all the plants of this species disappeared. *Neomeris annulata* formed a poor growth on the coral surface and it also had a short duration like *Acetabularia mœbii*.

By the beginning of April, a very complex community of green, brown and red algæ appeared on the coral surface, which persisted during May, June and even up to the middle of July, although there was always a tendency for *Padina gymnospora* to inhibit the growth of other algæ, particularly the greens, and even to replace them. In this competition, a very unsteady balance was attained among *Padina gymnospora*, *Sphacelaria tribuloides*, *Laurencia papillosa*, *Amphiroa fragilissima* and *Jania rubens*. By June, *Padina gymnospora* became few in number, and the other forms had almost started dwindling in population except *Laurencia papillosa*, which, though not very successful in the beginning in establishing itself along with the other algæ, was seen to have greater capacity to withstand the changing season; and it actually became luxuriant only when the other species became rare. From the close of September to the end of January, the boulder was mostly denuded except for a few forms; but during this period the coral surface was completely covered with *Lyngbya majuscula* harbouring a dense population of diatoms, particularly *Nitzschia closterium* and species of *Navicula*.

Table I shows the different species of algæ colonizing the coral surface of the boulder.

(*ii*) *Concrete faces.*—On the concrete faces, the colonization of the algæ was maximum on faces 2 and 4; lesser on face 3 and minimum on face 1. The seasonal fluctuation of the flora on the concrete faces was similar to that on the coral surface; but here, a greater number of species were represented. Remarkable among the forms observed on the concrete faces, as against the coral surface, were the members of Chlorophyta, particularly the species of *Caulerpa* and *Bœrgesenia forbesii*. Unlike on the coral surface, *Acetabularia mœbii* were fewer on the concrete faces; but many of these plants on the concrete faces were spared by the nudibranchs. *Neomeris annulata* showed better growth at greater depth. Those plants found toward the top of the boulder on the concrete faces were almost half the size of the ones found toward the base of the concrete faces, indicating, probably, lesser preference to light. The other forms, *Dictyota bartayresiana*, *D. maxima*, *Sargassum myriocystum*, *Hypnea valentiæ*, *Gracilaria lichenoides*, *Ceramium transversale* and *Laurencia obtusa*, although present on the concrete surface, were either a few or rare and of very short duration. Thus from a comparison of the species on the coral and the concrete surfaces, it would appear that the two kinds of surfaces do not present any vastly varying factors as far as the growth of marine algæ that inhabit calcarious substrates is concerned and that substratum selectivity does not come into question. Table II shows the different species of algæ colonizing the concrete faces of the boulder.

(*iii*) *Palmyra beams.*—The flora on the palmyra beams was rather poor, represented mostly by greens and blue-greens. Further, it was very surprising, that among the greens, while *Caulerpa peltata* grew abundantly on the beams, the other two species of *Caulerpa* were very poorly represented on them. In the case of blue-greens, *Lyngbya majuscula* found in abundance on the beams during March had only a short duration, and it again came into prominence during December and January, a few plants having been seen in the month of October and November. *Symploca hydnoides* was found in abundance early in April, and the plants continued their hold till late July; though, by the last week of May, the plants got reduced in population. During the month of March, when the blue-greens were in luxuriance on the beams, they harboured a very dense growth of *Climacosphænia elongata* and *Licmophora abbreviata*, and these diatoms were also found attached to *Hypnea valentiæ* growing on the beams. Table III shows the different species of algæ colonizing the palmyra beams.

TABLE I

Showing the different species of algæ colonizing the coral surface of the boulder

Algæ taking part in the colonization	Month of occurrence											
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ulva reticulata	..	..	..	F	R	..	R	..	R	..	..	..
Cladophora sp.	..	..	R	F	F	..	..	..	R	F	..	..
Chætomorpha media	F	M	..	..	..	..	..	..	..	..	..	..
Caulerpa peltata	..	..	..	..	..	..	..	..	..	..	..	..
Caulerpa racemosa	..	..	..	..	..	..	..	..	..	..	..	..
Caulerpa taxifolia	..	..	..	..	..	..	..	..	..	..	..	..
Acetabularia mœbii	..	..	M	M	R	..	..	..	..	..	..	..
Neomeris annulata	..	..	F	F	R	R	..	..	..	..	..	..
Bœrgesenia forbesii	..	..	..	..	..	..	..	..	..	..	..	..
Licmophora abbreviata	..	..	..	A	A	..	..	..	..	..	..	..
Climacosphœnia elongata	..	..	..	A	A	..	..	..	..	..	..	..
Cocconeis sp.	..	..	..	..	..	..	..	..	..	..	..	..
Navicula spp.	..	..	..	..	..	..	..	..	F	F	..	M
Nitzschia closterium	..	..	..	..	..	..	..	..	M	..	..	M
Sphacelaria tribuloides	..	..	..	F	M	A	A	R	..	..	..	..
Dictyota bartayresiana	..	..	..	..	..	..	..	..	..	..	..	..
Dictyota maxima	..	..	..	..	..	..	..	..	..	..	..	..
Padina gymnospora	F	A	D	A	M	F	F	R	R	R	R	M

Dictyota dichotoma	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Dictyota maxima	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Padina gymnospora	..	..	F	A	D	A	M	F	F	R	R	R	R	M
Sargassum myriocystum	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Liagora farinosa	..	..	..	..	..	..	..	..	..	R	..	..	..	..
Gelidium micropterum	..	..	..	..	..	..	..	..	R	..	..	..	..	..
Amphiroa fragilissima	..	..	..	..	..	A	D	M	M	R	R	..	..	..
Jania rubens	..	..	..	..	..	A	D	M	M	R	..	..	..	..
Lithophyllum sp.	..	..	R	R	R	R	..	..	..	..	..	..	..	..
Hypnea esperi	..	..	..	..	F	..	M	..	..	..	R	..	..	..
Hypnea valentiæ	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Gracilaria lichenoides	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Champia globulifera	..	..	..	..	M	M	R	..	..	..	..	..	..	..
Champia parvula	..	..	..	..	..	..	M	R	R	R	..	..	..	..
Chondrococcus hornemanni	..	..	..	..	..	..	..	..	..	R	..	..	..	..
Ceramium transversale	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Spyridia fusiformis	..	..	..	..	..	..	..	..	R	..	..	..	..	..
Roschera glomerulata	..	..	..	..	..	..	..	..	..	F	F	R	..	..
Acanthophora spicifera	..	..	..	F	..	..	..	..	R	..	..	..	..	..
Laurencia obtusa	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Laurencia papillosa	..	..	F	V	F	F	R	M	A	A	M	R	R	..
Leveillea jungermannioides	..	..	..	..	..	..	F	..	..	..	..	..	..	..
Lyngbya majuscula	..	..	D	F	R	..	M	..	..	..	..	M	V	D
Symploca hydnoides	..	..	..	..	..	..	..	..	..	..	..	..	..	..

D = Dominant; V = Very abundant; A = Abundant; M = Moderate; F = Few; R = Rare (less than 5 plants).

TABLE

Showing the different species of algæ colonizing the concrete faces of the boulder

Algæ taking part in the colonization	Month of occurrence											
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ulva reticulata	..	..	..	F	R	..	..	..	..	..	..	..
Cladophora sp.	..	..	..	F	M	..	..	..	F	..	..	..
Chætomorpha media	..	..	..	R	..	..	..	..	F	..	..	..
Caulerpa peltata	..	..	A	F	A	A	A	R	..	..	..	..
Caulerpa racemosa	..	..	..	F	M	..	..	..	..	..	..	..
Caulerpa taxifolia	..	..	..	F	..	..	..	..	..	..	..	..
Acetabularia mœbii	..	..	F	F	R	..	..	..	..	..	..	..
Neomeris annulata	..	..	M	M	F	..	..	..	..	..	..	..
Bœrgesenia forbesii	..	..	F	F	F	..	..	..	..	..	..	..
Licmophora abbreviata	..	..	..	A	A	..	..	..	..	..	..	..
Climacosphænia elongata	..	..	..	A	A	..	..	..	..	..	..	..
Cocconeis sp.	..	..	..	..	..	..	..	..	..	..	..	..
Navicula spp.	..	..	..	..	..	..	..	..	..	..	..	..
Nitzschia closterium	..	..	..	M	..	..	..	..	..	..	..	..
Sphacelaria tribuloides	..	..	..	R	M	M	M	R	..	..	..	..
Dictyota bartayresiana	..	F	..	R	..	M	..	..	..	..	..	..	..
Dictyota maxima	..	..	..	..	..	R	R	..	..	..	..	..	..
Padina gymnospora	..	..	F	A	A	A	M	F	F	R	R	R	R	R

Dictyota maxima							R	R	..	..	..	..	..	..
Padina gymnospora	F	A	A	A	M	F	F	R	R	R	R	R		
Sargassum myriocystum	..	..	..	..	..	..	..	R	R	R	..	..	..	
Liagora farinosa	..	..	..	..	..	..	..	..	..	..	..	..	..	
Gelidium micropterum	..	..	..	..	..	..	..	..	..	..	..	..	..	
Amphiroa fragilissima	..	..	..	..	A	V	M	..	..	..	..	..	..	
Jania rubens	..	..	..	..	A	V	M	..	..	..	..	..	..	
Lithophyllum sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	
Hypnea esperi	F	F	..	F	..	..	..	..	..	..	..	..	..	
Hypnea valentiæ	F	F	..	F	..	R	..	..	..	..	..	..	..	
Gracilaria lichenoides	..	..	..	R	R	R	..	..	R	..	..			
Champia globulifera	..	..	F	F	R	..	..	..	..	..	..	..	..	
Champia parvula	..	..	..	..	M	M	R	M	..	..	..	..	..	
Chondrococcus hornemanni	..	..	..	..	..	..	..	R	..	..	..	..	..	
Ceramium transversale	..	..	..	..	..	..	..	R	R	..	..	..	..	
Spyridia fusiformis	..	..	..	..	F	..	F	..	..	..	..	..	..	
Roschera glomerulata	..	..	..	..	..	..	..	F	F	R	..	..	..	
Acanthophora spicifera	..	A	..	..	..	..	R	..	..	..	..	..	..	
Laurencia obtusa	..	..	..	..	..	R	R	..	..	..	..	..	..	
Laurencia papillosa	..	..	F	F	M	M	R	R	..	..	..	..	..	
Leveillea jungermannioides ..	..	..	..	..	F	..	..	..	..	..	..	..	..	
Lyngbya majuscula	V	F	..	..	A	..	..	..	..	M	V	V		
Symploca hydnoides	..	..	..	..	..	..	..	..	..	..	..	..	..	

D = Dominant; V = Very abundant; A = Abundant; M = Moderate; F = Few; R = Rare (less than 5 plants).

TABLE III

Showing the different species of algæ colonizing the palmyra beams

Algæ taking part in the colonization	Month of occurrence											
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ulva reticulata	..	..	..	..	..	..	..	..	..	..	..	..
Cladophora sp.	..	..	..	..	F	..	..	..	..	..	..	..
Chætomorpha media	..	..	..	..	..	..	..	..	F	..	..	..
Caulerpa peltata	..	..	..	V	F	A	A	F	..	..	..	..
Caulerpa racemosa	..	..	..	F	F	..	..	..	..	..	..	..
Caulerpa taxifolia	..	..	..	F	F	..	..	..	..	..	..	..
Acetabularia mœbii	..	..	..	..	..	..	..	..	..	..	..	..
Neomeris annulata	..	..	..	..	..	..	..	..	..	..	..	..
Bœrgesenia forbesii	..	..	..	..	..	..	..	..	..	..	..	..
Licmophora abbreviata	..	..	..	..	..	..	..	..	..	..	..	..
Climacosphænia elongata	..	..	..	..	A	..	..	..	..	..	..	..
Cocconeis sp.	..	..	..	..	A	..	..	..	..	..	..	F
Navicula spp.	..	..	..	..	..	..	..	..	..	..	..	F
Nitzschia closterium	..	..	..	..	..	..	..	..	..	..	..	..
Sphacelaria tribuloides	..	..	..	..	..	..	..	..	..	..	..	..
Dictyota bartayresiana	..	..	..	..	..	..	..	..	..	..	..	..
Dictyota maxima	..	..	..	..	..	F	..	..	..	..	..	..

Dictyota maxima	..	..	..	..	..	..	..	..	F	..	..	..	..	..	..
Padina gymnospora	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Sargassum myriocystum	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Liagora farinosa	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Golidium micropterum	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Amphiroa fragilissima	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Jania rubens	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Lithophyllum sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Hypnea esperi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Hypnea valentiæ	..	..	..	..	..	..	..	F	..	..	..	..	..	..	..
Gracilaria lichenoides	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Champia globulifera	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Champia parvula	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Chondrococcus hornemanni	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ceramium transversale	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Spyridia fusiformis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Roschera glomerulata	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Acanthophora spicifera	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Laurencia obtusa	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Laurencia papillosa	..	..	..	..	..	F	F	M	R	..	..	..	..	..	..
Leveillea jungermannioides	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Lyngbya majuscula	..	..	..	A	..	..	..	A	..	..	..	..	F	F	M
Symploca hydnoides	..	..	..	..	..	..	A	V	F	F	..	..	..	..	..

D = Dominant; V = Very abundant; A = Abundant; M = Moderate; F = Few; R = Rare (less than 5 plants).

BIOTIC FACTORS

During the above studies, the boulder was found to be harbouring gastropods, nudibranchs, crabs, hydroid colonies and sponges. The hydroid colonies and sponges were found to be abundant on the palmyra beams and in fact they were the pioneer colonizers, the algæ having appeared much later. Gastropods were found all through the season; while nudibranchs were only of a short duration when there was a good growth of green algæ on the coral surface, particularly *Acetabularia mœbii*. Crabs were very often present in large numbers when *Padina gymnospora* plants were in full growth. Most of these organisms were hand-picked and removed from the boulder and palmyra beams during periodic inspections of the boulder.

GROWTH RATE

Among the algæ found common on the coral and the concrete surfaces, the ones on the latter had more luxuriant growth except *Amphiroa fragilissima* and *Jania rubens* which were more abundant and luxuriant on the coral surface. Further, in general, the early growth of all the forms was more marked on the coral face than on the concrete faces; but, after a particular stage, all the forms on the concrete faces developed faster and became more robust. This applies to those found growing even on concrete face 1, although here the number of species represented was much limited compared to the other faces. At the height of the growing season, the limits of size achieved before reproduction took place were still further extended. Thus, on the coral surface, in the case of *Padina gymnospora*, many plants with fronds less than 3 cm., were seen with mature tetrasporangia; while, on the concrete faces the plants generally produced tetrasporangia after attaining a height of 5 cm., or more. Again, on concrete face 1, *Padina gymnospora* plants were seen to be much better developed with greater number of fronds from the same base; while on the other concrete faces and on the coral surface, plants with one or two fronds were met with more often. In other words, for a luxuriant growth, *Padina gymnospora* prefers a sheltered place rather than regions exposed to wave action. During the unfavourable season, from the end of July to November, only few *Padina gymnospora* plants were recorded; and all of them, without any exception, were subsequent growths from older plants which were broken off from the holdfasts or eaten off by grazing animals. In this vegetative growth it has been observed that the fronds were very coarse and smaller compared to fronds produced by sporelings. In the case of *Sphacelaria tribuloides*, *Amphiroa fragilissima* and *Jania rubens*, in the initial stage, the plants were very fast growing, extending over a period of two to three weeks, after which, the growth was seen only

in the lateral spread of the plants resulting in a bushy nature. In *Sphacelaria tribuloides*, the maximum height recorded was 1·8 cm., in plants which were about ten weeks old. In the course of six weeks, *Amphiroa fragilissima* and *Jania rubens* attained a maximum height of 4·3 cm. and 2·7 cm., respectively. The growth of *Laurencia papillosa* was rather difficult to follow, because of the fact that the plants were fast growing ones; and in four weeks time, from sporelings, they attained a height of more than 8 cm. But in the majority of cases, it was observed that when the plants attained a height of about 4–6 cm., most of the branches broke off at the places of origin and even the main thallus broke off leaving small stumps with few broken branches. Fresh branches were seen to arise from these stumps resulting in a denser growth. Stumps which had put forth branches two or three times were observed to be stunted and the subsequent branches produced by them were very small with profuse fruit bodies.

In general, most of the growth activities of the algæ were over in three to ten weeks, except in the case of *Caulerpa*, *Dictyota*, *Sargassum*, *Lithophyllum*, *Gracilaria* and *Acanthophora*. In *Caulerpa*, the growth was a continuous process all through the favourable season; and as the plants continued growth and spread on the substratum, the older regions became yellowish and rotted. When such rotting was of a high order, it was observed that these rotten regions were soon colonized by hydroid colonies and sponges. All the other forms of algæ above-mentioned were ill-represented on the boulder and hence no proper growth rate measurements could be taken; and in the case of *Sargassum myriocystum* almost all the plants got detached from their hold even when they were less than 15 cm. tall. This is partly because plants of *Sargassum myriocystum* were seen to make their appearance on the boulder during the rough season, and probably also because the concrete surfaces were not the proper substrata for their firm anchorage.

It has been observed earlier, that there is not much of substratum selectivity exhibited by the algæ where concrete and coral substrata are in question. Therefore the growth rate observations indicate that the algæ in general prefer a vertical to a horizontal substratum; position perhaps acting along with the microclimate. However, this is applicable only to the smaller forms and not to the larger ones like *Gracilaria* and *Sargassum*.

Reproductive Phase

In the case of *Cladophora* sp., early in the month of April, almost all the plants showed the presence of empty apical cells with apical openings indicating the possibility of having produced swarmers, although such

swarmers were not actually met with. *Sphacelaria tribuloides* propagated only vegetatively by means of the propagules, and sporangia were not observed. In the case of *Padina gymnospora* only tetrasporic plants were observed on the boulder; the production of tetraspores were very much earlier on the coral surface than on the concrete faces. The tetraspores were produced at very regular intervals of about two weeks, but the tetrasporic zones were better developed only during the months of March, April and May. There was practically very little spore production during August to November. In both *Amphiroa fragilissima* and *Jania rubens*, mature conceptacles were seen in abundance during late April and late June. *Hypnea valentiæ* produced only tetrasporic plants, and tetrasporangia were seen in plants of all age-group, they being produced more often in a regular sequence from the base of the plant upward, except in few cases where the nemathecia were seen scattered even at the terminal parts of the plants. *Laurencia papillosa* also appears to reproduce by spores, although sporelings or spores were not met with in the water samples collected from the small crevices on the coral surface; but, cystocarps were seen in abundance during the middle of June and during the last week of November.

In all the above cases, it has been observed that during the reproductive season, both fertile and sterile plants of the same size-group existed side by side. Such a co-existence of fertile and sterile plants suggests that the formation of reproductive bodies is not entirely dependent on environmental factors, but is essentially controlled by an internal factor of the individual plant which factor interacting with the environmental conditions results in the reproductive processes following a period of growth.

CHEMICAL FACTORS

An attempt at a correlation of the chemical factors of the environment with the growth and reproduction of the algæ is apparently far-fetched. Some investigators as Klebs (1896), hold the view that environmental factors are of primary importance in inducing the formation of reproductive bodies, but when taken as a whole, investigations of this type are mostly contradictory. Broadly speaking, maximum vegetation and growth are seen during the months when the salinity of the sea-water is moderate. From August, the flora gets depopulated with practically very few species occurring during the months of November to January and even early February, when the salinity fall is too rapid with, probably, marked fluctuations. Exception to this is the blue-green algæ, *Lyngbya majuscula* and *Symploca hydnoides* which appear to tolerate and prefer a steep fluctuation in salinity.

Considering total nitrogen of the water, most of the forms appeared during the peak period, between February and March, but as they became luxuriant, the total nitrogen content of the water indicated a fall; and it was mostly during the low period that the reproductive structures were seen to develop. The rhythmic fluctuation in total nitrogen seems to correspond with the periodic vegetative and reproductive phases of the algæ in general.

Consideration of the temperature as a contributory factor has a very limited scope, since the temperature range is very small. But, here again, in general, the majority of forms were seen to flourish well during the warmer months and the frequency of sporulation was faster and of shorter duration during these seasons.

Other Dynamic Factors

Apart from the fact that the turbulence of the water acting in an indirect manner in modifying the physical and chemical quality of the water, it was observed that when the sea was rough and turbulent, most of the smaller and delicate algæ got completely detached from their hold or got torn off the base. In the above studies, an exception seen was in the case of *Lyngbya majuscula*, which was seen to withstand the turbulence of the sea.

Relation of the Surrounding Flora on the Colonization

From periodic observations of the immediate vicinity of the experimental boulder, it has been observed that the majority of the algæ found on the boulder were seen growing in the immediate vicinity also. But *Acanthophora spicifera*, *Hypnea valentiæ*, *Ulva reticulata* and *Gracilaria lichenoides*, abundant all around in Palk Bay, were very poorly represented on the boulder. The same is applicable in the case of *Liagora farinosa*, although this alga was not in such abundance in the immediate vicinity. On the contrary, *Padina gymnospora* was not found to any appreciable level in the surrounding area, but was seen all year round on the boulder. This is applicable in the case of the blue-green algæ also, which were never in abundance in the immediate vicinity. It was also observed that during certain seasons, Palk Bay had a dense growth of sea-grasses, particularly *Cymodocea* spp., and *Halophila* spp., on which plenty of *Chætomorpha linum* (Müller) Kützing, were seen growing entangled. But at no time, on the boulder, were sea-grasses or *Chætomorpha linum* seen.

Summary

Succession of marine algæ on a fresh substratum—a concrete boulder with the top surface paved with coral stones—was studied. The algæ

colonizing the boulder were identified; the pioneer communities, succession, biotic factors, growth rate and reproductive phases of some of the forms, chemical factors, other dynamic factors and relation of the surrounding flora on the colonization over a period of 18 months were noted.

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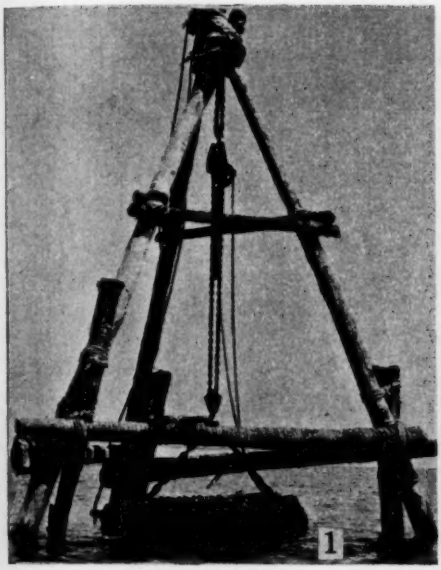

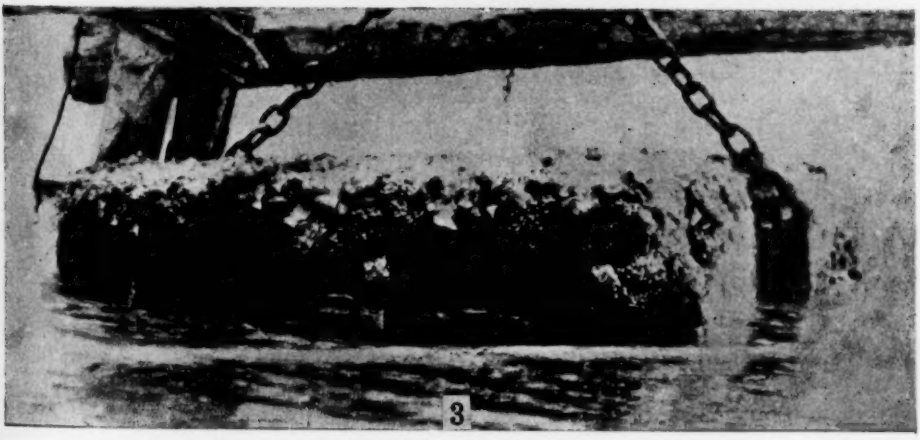

FIGS. 1–3

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Explanation of Plate IV

Fig. 1. Showing the leverage-tripod in position around the boulder which is partly lifted up by chain blocks.

Figs. 2 and 3. Showing the luxuriant growth of *Padina gymnospora* on the coral surface durin g February to April.

THE EFFECT OF INSULIN ON THE PROTEIN-BOUND AMINO ACIDS OF THE PITUITARY OF THE FISH—*OPHICEPHALUS STRIATUS* BLOCH.

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Introduction

Ever since Banting's discovery of the insulin function of the pancreas, the physiological effects of insulin on carbohydrate metabolism have been the subject of numerous investigations. It is only in comparatively recent years that evidence has been accumulating to show that insulin promotes protein metabolism. A number of investigators have studied the effect of hypoglycæmia on the amino acids of the tissues and organs, in particular, blood, liver and muscle. Dawson (1950) found that in hypoglycæmia in rats, produced by insulin injection, the glutamic acid content of the brain was reduced below the normal level.

The investigations on the effect of insulin injection have so far been almost exclusively in mammals. Only in very few cold-blooded animals have such studies been carried out. These include the effect of insulin on fishes with respect to changes in the physiological conditions (Brunn and Hemmingsen, 1938) and distribution of glycogen and blood sugar (McCormic and MecLeod, 1925; Grey, 1928; Grey and Hall, 1930; Root *et al.*, 1931 and Vorhauner, 1938). Root *et al.* (1931) who studied the influence of insulin injection on the glycogen distribution in marine fishes have pointed out that, due to slower metabolism, a poikilothermal animal is more favourable for the study of insulin action "....as the results are brought to us in the form of a slow motion picture and details of action can be studied with great facility".

I have been studying the effect of insulin on the carbohydrate and protein metabolism in the common fish *Ophicephalus striatus* Bloch. The present account deals with the effect of hypoglycæmia on the protein-bound amino acids of the pituitary gland of this fish. The interrelationships of the hypophyseal hormone and insulin are fairly well known. But the investigations

that have been made so far by several endocrinologists relate to the effect of the pituitary on insulin, and not the effect of insulin on the pituitary which forms the subject of the present study. In view of the critical importance of the pituitary in the endocrinal regulation and the co-ordination of the bodily function, it is rather surprising that no investigation seems to have been made so far on the effect of insulin injection on the pituitary.

Material and Methods

In the present study the effect of insulin injection on the protein-bound amino acids of the pituitary (except tryptophane) was investigated by paper chromatography. Both qualitative and quantitative determinations of the amino acids of the pituitary were made at intervals of 0·5, 1·0, 1·5, 2·0 and 2·5 hours, following the injection of insulin.

Specimens of *Ophicephalus striatus*, weighing about 150–200 gm. each, and always of about the same stage and growth of maturity of the gonads, were injected with 5 units of insulin. The pituitary glands were dissected out in the fresh living condition, carefully avoiding extra tissues and placed immediately in 6 N HCl. The extracts were prepared in accordance with the procedure adopted by Giri *et al.* (1952 *a* and *b*). The samples were hydrolysed for eighteen hours under reflux in a water-bath at 100° C. After completion of the hydrolysis the contents were evaporated several times using distilled water to remove all the traces of the acid used. After the hydrolysate was finally concentrated to dryness, a few drops of 10 per cent. isopropyl alcohol were added to serve as a preservative. Known micro quantities of extracts were used for spotting after noting down the final volume of the extract.

Circular chromatograms were run using Whatman No. 1 filter-paper with diameter of 12·5 cm. The amino acids were identified by running mixed chromatograms with both the extract and known amino acids. Two dimensional chromatograms for both known and unknown were run under identical conditions using Whatman No. 1 with 25×25, and 20×20 cm. dimensions. Butanol: acetic acid: water (4: 1: 1) and phenol saturated with buffer were used as solvents (Block, 1952). By this method all the overlapping amino acids were clearly separated. For quantitative estimations the spots of the individual amino acids were cut out and eluted in 4 ml. of 75 per cent. alcohol containing 0·4 ml. of 0·1 per cent. copper sulphate solution (Giri *et al.*, 1953). The colour intensities were determined colorimetrically with Lumetron Model No. 401 A, with 550 mμ filter. The quantities of amino acids are expressed in micrograms per pituitary gland (with a mean weight

of 0·002 gm.). As the pituitary glands are very small and as their mean weight shows little or no significant variation, the quantities of the amino acids are expressed per pituitary gland.

RESULTS

The pituitary of normal or untreated fish (*Ophicephalus striatus*) has the following twelve amino acids: (1) Leucines, (2) Valine, (3) Tyrosine, (4) Alanine, (5) Threonine, (6) Glycine, (7) Serine, (8) Glutamic acid, (9) Aspartic acid, (10) Histidine, (11) Lysine and (12) Ornithine.

The effect of insulin injection is seen on all amino acids. But the degree and duration of the effect varies with different amino acids. The effects may be discussed under the following headings: (1) Qualitative effects, (2) Quantitative effects and (3) Recovery from insulin effect.

TABLE I

The effect of insulin on the protein-bound amino acids of pituitary of Ophicephalus striatus *Bloch.*

(Qualitative Analysis)

Amino acids	Normal	0·5 hour	1·0 hour	1·5 hours	2·0 hours	2·5 hou
Leucines ..	+	+	−	−	−	+
Valine ..	+	+	−	+	+	+
Tyrosine ..	+	+	−	−	+	+
Alanine ..	+	+	+	−	+	+
Threonine ..	+	+	+	+	+	+
Glycine ..	+	+	+	+	+	+
Serine ..	+	+	+	+	+	+
Glutamic acid ..	+	+	+	+	+	+
Aspartic acid ..	+	+	−	−	+	+
Histidine ..	+	+	+	+	+	+
Lysine ..	+	+	+	+	+	+
Ornithine ..	+	+	+	+	+	+

Qualitative effects.—This is shown in the chromatogram (Fig. 1). This is seen only in respect of leucines, valine, tyrosine aspartic acid and alanine. All these five amino acids disappear but not at the same time. Leucines, valine, tyrosine and aspartic acid disappear at one hour after injection, and alanine at 1·5 hours after injection. The duration of the effect of insulin lasts from 1·0–2·0 hours in the case of leucines, for aspartic acid and alanine from 1·0–1·5 hours after injection. For valine the disappearance is restricted to 1·0 hour stage and for alanine to 1·5 hours stage only.

Quantitative effects.—The injection of insulin effects all the amino acids quantitatively and this is noticeable from the very commencement of the observation. But the degree and duration of the effect varies for the different amino acids. Since leucines, valine, tyrosine and aspartic acid disappear

TABLE II

The effect of insulin on the protein-bound amino acids of pituitary of Ophicephalus striatus *Bloch.*

(Quantitative Analysis)

(Amino acids are expressed in micrograms per pituitary gland)

Amino acids	Normal	0·5 hour	1·0 hour	1·5 hours	2·0 hours	2·5 hours
Leucines ..	10·20	7·49	..	..	..	5·62
Valine ..	15·62	11·89	..	3·47	6·12	10·71
Tyrosine ..	6·12	4·75	..	..	5·02	6·79
Alanine ..	12·41	8·13	6·37	..	5·36	10·49
Threonine ..	18·52	16·00	14·97	2·13	3·78	6·64
Glycine ..	52·93	45·87	30·26	15·62	26·91	42·78
Serine ..	26·21	24·54	13·60	9·78	15·91	17·53
Glutamic acid ..	72·45	38·15	16·18	7·68	21·32	45·48
Aspartic acid ..	42·46	12·94	..	..	9·32	20·68
Histidine ..	16·35	12·58	9·65	4·91	10·35	15·68
Lysine ..	20·69	18·13	5·41	3·12	8·61	18·05
Ornithine ..	10·45	8·24	3·69	2·63	5·35	8·42

completely at 1·0 hour stage, the insulin effect is represented in the table as 100 per cent. fall of the amino acids (Table III). Similarly for alanine the 100 per cent. fall is at 1·5 hours.

TABLE III

The effect of insulin on the protein-bound amino acids of pituitary of Ophicephalus striatus *Bloch.*

(Percentage of fall and recovery)

Amino acids	Fall					Recovery
	0·5 hour	1·0 hour	1·5 hours	2·0 hours	2·5 hours	
Leucines ..	26·6	100·0	100·0	100·0	44·9	55·1
Valine ..	23·9	100·0	77·8	60·8	31·4	68·6
Tyrosine ..	22·4	100·0	100·0	18·0	10·9 (increase)	..
Alanine ..	34·5	48·8	100·0	56·8	15·4	84·6
Threonine ..	13·6	19·1	88·5	79·5	64·0	36·0
Glycine ..	13·3	42·8	70·5	48·1	19·2	80·2
Serine ..	6·4	48·1	62·7	39·3	33·1	66·9
Glutamic acid ..	41·8	77·6	89·4	70·5	37·2	62·9
Aspartic acid ..	69·3	100·0	100·0	78·1	51·3	48·7
Histidine ..	23·1	41·0	70·0	36·7	42·0	58·0
Lysine ..	12·4	73·8	84·9	58·4	12·8	87·2
Ornithine ..	21·2	64·7	74·8	48·8	19·4	80·6

The duration of the effect of leucines, as stated already, is from 1·0–2·0 hours, for tyrosine and aspartic acid 1·0–1·5 hours, and for valine and alanine limited each to one stage only.

The degree of the insulin effect is indicated in Table III by percentages of fall of different amino acids at different intervals. At 0·5 hour after injection all the amino acids show a decline. This decline increases at one hour, and there is an overall maximum fall at 1·5 hours, when none of the amino acids has less than 62·7 per cent. fall compared with the normal amino acids content. But the time of the maximum fall for the individual amino acids varies in respect of those which disappear completely as explained

above. Leucines, valine, tyrosine and aspartic acid have 100 per cent. fall at one hour following injection and alanine at 1·5 hours.

Recovery phase.—The onset of recovery phase and the degree of recovery vary with different protein-bound amino acids. This has been alluded to already in the case of the amino acids which disappear completely. The recovery from the insulin effect for valine which disappears at 1·0 hour begins at 1·5 hours. For leucines the recovery commences only 2·0 hours. The other amino acids commence their recovery at 2·0 hours. But the recovery is not complete even at 2·5 hours for any amino acid except tyrosine, which seems to show a slight increases over its normal content in the pituitary. Whether this slight rise is really significant has to be further investigated.

The degree of recovery for various amino acids also varies. For alanine, glycine, lysine and ornithine, the recovery is 80 per cent. and slightly over. For valine, serine and glutamic acid, the recovery is from 62·9–68·6 per cent. Histidine and leucine recover by less than 60 per cent. but above 50 per cent., and threonine and aspartic acid by less than 50 per cent. but more than 35 per cent.

Discussion

Dawson (1950) investigated the effect of insulin on the free amino acids of the brain of the rat and found that the content of L-glutamic acid was reduced as a result of hypoglycæmia following insulin injection. He suggested that this reduction in glutamic acid is due to its oxidation and also demonstrated that it is not related to release of adrenalin evoked by hypoglycæmia.

In the present investigation the effect of injection of insulin on the protein-bound amino acids of a cold-blooded animal has been investigated and the results show that all the amino acids are affected quantitatively and some qualitatively. This would show that the injection of insulin has a profound effect on the amino acid constitution of the pituitary and thus on the protein metabolism. Further work is in progress.

Summary

The effect of insulin injection on the protein-bound amino acids (except tryptophane) of the pituitary of *Ophicephalus striatus* has been studied chromatographically at intervals of 0·5, 1·0, 1·5, 2·0 and 2·5 hours. It is found that all the protein-bound amino acids are affected quantitatively. Leucines, valine, tyrosine, aspartic acid and alanine disappear completely for sometime as a result of insulin injection. The other amino acids show varying degree of reduction. The recovery of all amino acids expecting tyrosine is not complete even after 2·5 hours. The degree and duration of recovery varies for different amino acids.

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EXPLANATION OF PLATES

PLATE V

FIG. 1. Chromatogram showing the effect of insulin injection on the protein-bound amino acids of pituitary of *Ophicephalus striatus* Block.

N: Protein-bound amino acids of pituitary of normal animal.

0·5, 1, 1·5, 2, 2·5: Protein-bound amino acids of pituitary at the end of 0·5, 1·0, 1·5, 2·0 and 2·5 hours after insulin injection.

(1) Leucines, (3) Valine, (5) Tyrosine, (7) Alanine, (8) Glutamic acid—Threonine, (10) Glycine—serine—aspartic acid, (13) Histidine, (14) Lysine—Ornithine.

PLATE VI

FIG. 2. Histogram showing the quantitative variations in the protein-bound amino acids content of Pituitary of *Ophicephalus striatus* Bloch., after the injection of 5 units of insulin at intervals of 0·5, 1·0, 1·5, 2·0 and 2·5 hours.

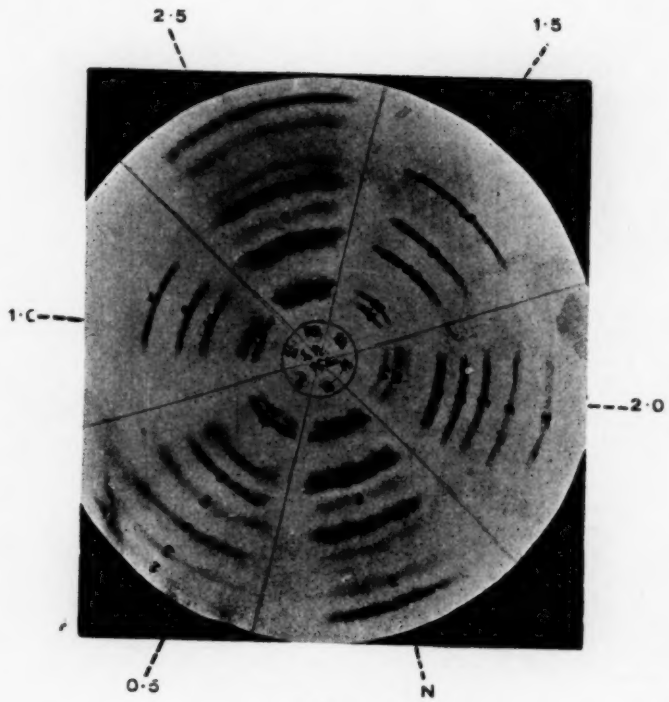

FIG. 1

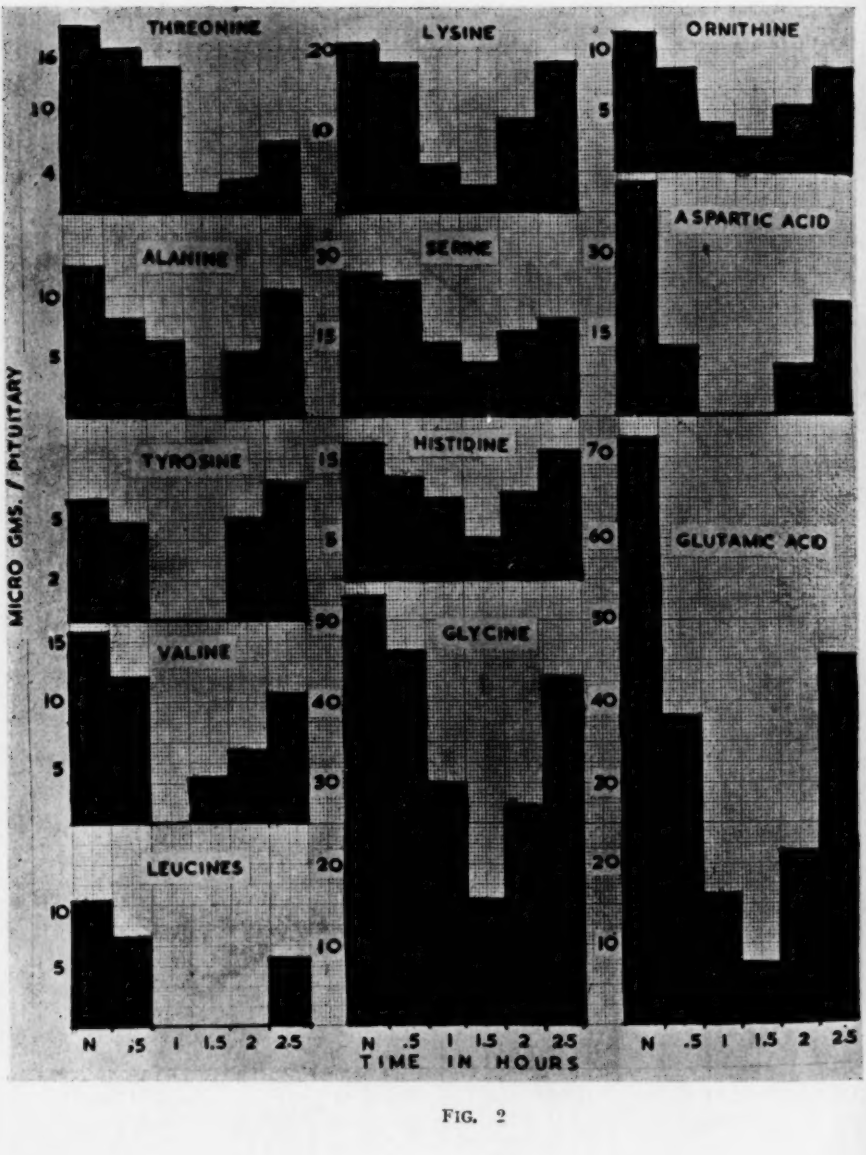
THREONINE
LYSINE
ORNITHINE
ALANINE
SERINE
ASPARTIC ACID
TYROSINE
HISTIDINE
GLUTAMIC ACID
VALINE
GLYCINE
LEUCINES
MICRO GMS. / PITUITARY
N .5 1 1.5 2 2.5
TIME IN HOURS

FIG. 2

LIGHT PENETRATION IN THE INSHORE WATERS OF PORTO NOVO

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(Communicated by Prof. R. V. Seshaiya, F.A.SC.)

Introduction

It is generally recognised that the intensity and composition of light play an important role in the distribution of planktonic organisms in the sea. The production of planktonic plant life is confined to the upper illuminated area or the euphotic zone and the amount of production and times of onset of the phytoplankton outburst is dependent upon the illumination. Knowledge of solar radiation at different depths is thus of great importance to the marine biologist. To the physical oceanographer the study of solar radiation affords a means of computing the rate at which the radiant energy from the sun and sky is absorbed and converted into heat in the uppermost layers of the hydrosphere and thus calculate the ensuing rise in temperature.

Previous Work

The Swedish oceanographers have studied the subsurface radiant energy and the transparency of the sea. The first measurements of subsurface radiation were made by Fol and Sarasin in 1885 by exposing photographic plates at different depths in the Mediterranean. Atkins and Poole (1933) have done pioneer work at sea by using the selenium rectifier cells. Some measurements have been recorded in North Pacific Ocean by Utterback (1936) and in Mid Atlantic Ocean by Oster and Clarke (1935). The observations made so far are relatively few, and none appears to have been made in Indian coastal waters. Recently, however, Satyanarayana Rao (1958) published data on the transparency of waters on the East Coast of India taken with the Secchi disc. The Secchi disc method gives only the depth of visibility and thus indirectly a measure of transparency. Though a useful method, it cannot be an accurate one, as visibility is affected by absorption due to water itself or substances dissolved in it, *i.e.*, its color, and by scattering due to turbidity. The direct method of measuring the transparency of the sea with the aid of photoelectric cells has the greatest advantage and eliminates these

errors. The transparency of Indian waters has not so far been investigated with the aid of the photoelectric cells.

Jerlov (1951) has remarked that the Indian Ocean in March and April before the monsoon presents day after day ideal weather with a smooth sea and cloudless sky, so that here most problems connected with the submarine daylight could be attacked.

MATERIAL AND METHOD

The object of the present paper is to report the results of preliminary observations on the measurements of submarine daylight carried out by means of photoelectric method *in situ* at the 5 fathoms line in the sea at Porto Novo. The small photometer which was used for taking measurements in the present investigation is of the type previously described by Petterson and Poole (1937). The photometer was suspended by a bridle so that the window was kept horizontal. The diffusing layer of the opal glass was always kept internal so that the diffusing properties were not affected by immersion in water. The diffusing glass was always mounted above the color filter. The window of the photometer has a diameter of 50 mm. and the various color filters used have a thickness of 2·0 mm. The space between the window of the photometer and the color filter as well as between the color filter and the opal glass was filled with sea-water before taking measurements. The details for the color filters used for taking measurements taken from Jerlov (1951) are given below:

Filter No.	Code number of filters used	Width in mμ	Centre of gravity in mμ
1	UG 1+BG 12	30	375
2	BG 12	80	435
3	GG 5+BG 12	40	465
4	GG 5	50	475
5	OG 2	50	590
6	RG 1	25	625
7	RG 5	20	680

The photometer was suspended by a cable passing over a winch and kept clear of the shadow of the vessel and the other end of the cable was connected

to a small 'Lux meter' having a full-scale deflection of 500 Lux units. Readings were taken on the deck, above the surface of water and at every meter depth below the surface. All measurements were carried out at noon local time with a clear sun. For measuring the reflected light the photometer was kept turned upside down. To study the upwardly scattered component of sunlight the photometer was directed downwards and kept just below the surface of the water when measurements were taken.

The vertical extinction coefficient is the rate at which downward travelling radiation decreases, *i.e.*, the percentage of radiation held back in one meter of water. The vertical extinction coefficient, K, was calculated using the formula $I/I_0 = e^{-KL}$, where the light is reduced from I_0 to I by a substratum L meters thick.

Observations

Percentage of surface radiation at different depths.—The percentages of surface radiation at various depths for different wavelengths of daylight are represented in Figs. 1 and 2. Wavelengths below 475 mμ are represented in Fig. 1 and wavelengths above 475 mμ in Fig. 2. Nearly half of the total amount of near ultra-violet radiation available at the surface is absorbed by first one meter of water. At one meter depth the near ultra-violet

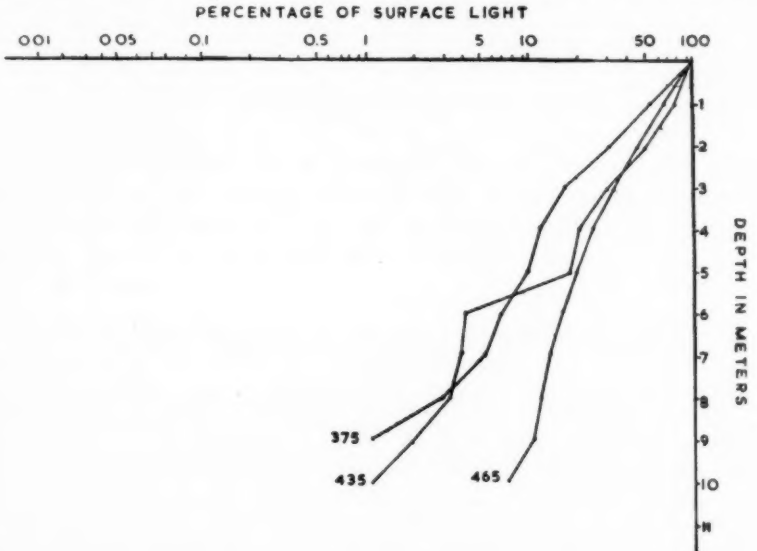

Fig. 1. **Percentage of surface radiation at different depths for wavelengths 375, 435 and 465 mμ.**

radiation is only 51·8 per cent. Blue light with a wavelength of 435 mμ is least absorbed by the surface layers. The percentage of surface radiation with 435 mμ at 1 meter depth is 77·4 per cent. Only about 23 per cent. of light is absorbed by the first one meter.

An analysis of the data shows that solar energy of 435–475 mμ has the highest percentage of penetration at various depths. Red light with a wavelength of 680 mμ shows a very heavy absorption. Nearly 96 per cent. of the surface radiation of 680 mμ is absorbed by the surface layer. At a depth of 7 meters the red part of the spectrum has complete extinction. Blue light of 435 mμ penetrates a little further than the red, showing extinction at 11 meters.

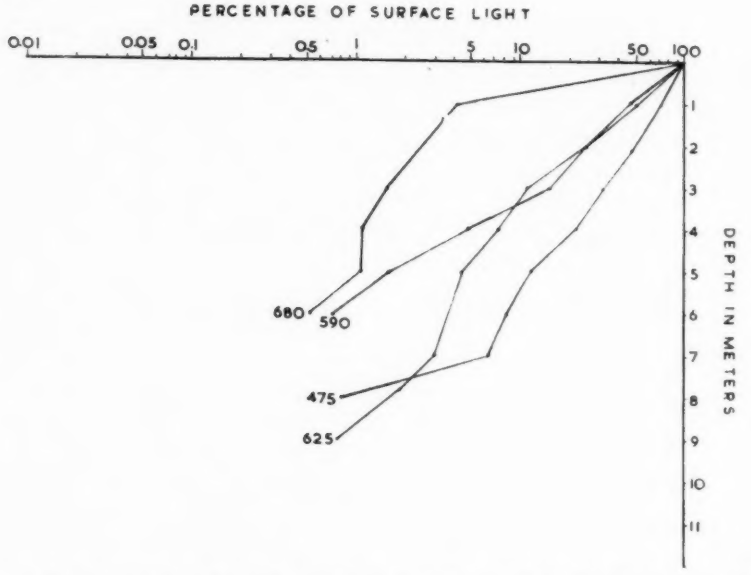

FIG. 2. Percentage of surface radiation at different depths for wavelengths 475, 590, 625 and 680 mμ.

Figure 3 represents the depths at which the percentage of surface radiation is 10 per cent. and 1 per cent. for different wavelengths. It is clear from the graph that radiations at 465 mμ penetrates farther into the sea, and radiations at longer wavelengths are heavily absorbed by the surface.

Vertical extinction coefficient.—The vertical extinction coefficient for different wavelengths is shown in Table I. The value of extinction coefficients varies from 0·25–1·0 per meter for 375 mμ, 0·05–1·49 per meter for 435 mμ

and 0·34–1·11 per meter for 590 mμ. Red light of 680 mμ shows a highest value of K for the first one meter of water, the value being 3·17. Blue light with a wavelength of 345 mμ has the lowest extinction coefficient of 0·26 for the first one meter of water.

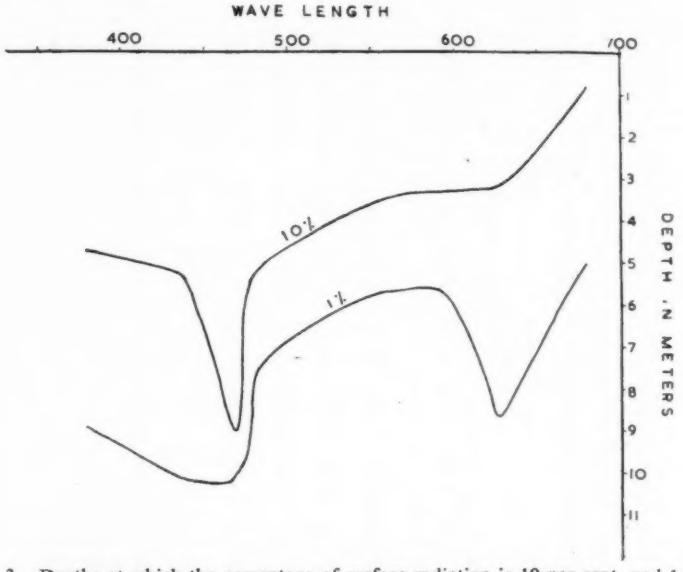

FIG. 3. Depths at which the percentage of surface radiation is 10 per cent. and 1 per cent. for different wavelengths.

Jorgensen and Utterback (1939) working in the inshore waters of Pacific Northwest have recorded extinction coefficients varying from 0·48–0·83 per meter for the wavelength 660 mμ. In the inshore waters of Porto Novo the value of extinction coefficient for wavelength 680 mμ varies from 0·37–3·17 per meter.

Upwardly scattered component of sunlight.—The incident, reflected and scattered light were calculated by applying the formula $I = A + R + U$, where I is the incident light available at the surface, A the part of light that penetrates the sea surface and is absorbed, $R + U$ the total light coming upward from the sea surface and U the light that penetrates the sea surface but is scattered back by the particles and bubbles in the water below and finally returns to the air. It is found that high scattering occurs in the wavelengths 475–590 mμ. Red light of wavelength 680 mμ is not at all scattered and 99·5 per cent. of the light is absorbed by the water. Jorgensen and Utterback (1939) have recorded that 1–3 per cent. of light is scattered in the

TABLE I

Vertical extinction coefficient for various wavelengths at different depths in meters

No.	Filter	Wave-length mμ	Depth in meters									
			1 m.	2 m.	3 m.	4 m.	5 m.	6 m.	7 m.	8 m.	9 m.	10 m.	11 m.
1	UG 1+BG 12	375	0·66	0·56	0·57	0·34	0·25	0·33	0·33	0·47	1·00	0·00	..
2	BG 12	435	0·26	0·43	0·55	0·35	0·11	1·49	0·05	0·20	0·37	0·69	0
3	GG 5+BG 12	465	0·43	0·37	0·32	0·26	0·22	0·19	0·16	0·12	0·13	0·38	0
4	GG 5	475	0·35	0·39	0·39	0·35	0·60	0·41	0·28	1·62	0·00	..	..
5	OG 2	590	0·76	0·59	0·51	1·09	1·11	0·34	0·00	..	..	..	..
6	RG 1	625	0·73	0·62	0·85	0·41	0·52	0·17	0·21	0·46	0·44	00·0	..
7	RG 5	680	3·17	0·48	0·49	0·37	0·37	0·00	..	..	..	..	..

TABLE II

The ratio between upward and downward illumination U/V per cent.

No.	Filter	Above surface	Depth in meters								
			1 m.	2 m.	3 m.	4 m.	5 m.	6 m.	7 m.	8 m.	9 m.
1	UG 1+BG 12	1·5	0·7	0·0	..	..	..	..	..	..	..
2	BG 12	3·2	2·8	3·8	5·9	5·3	5·9	26·3	11·1	0·0	..
3	GG 5+BG 12	5·0	3·8	5·6	4·6	4·0	2·5	0·0	..	..	..
4	GG 5	6·3	5·9	7·0	·103	9·1	6·7	6·7	0·0	..	..
5	OG 2	6·9	5·9	6·7	6·7	6·7	0·0	..	..	..	..
6	RG 1	1·9	0·0	..	..	..	..	..	..	..	..
7	RG 5	0·5	0·0	..	..	..	..	..	..	..	..

short wavelength and 0·5–2·0 per cent. in the long wavelength in the coastal waters of San Juan islands. The observations made so far in the coastal waters of Porto Novo show that as high as 5·9 per cent. of light is scattered in the wavelength 475–590 mμ. The high percentage of scattering in the coastal waters of Porto Novo is evidently due to the presence of large quantity of suspended particles carried by the Vellar river into the sea.

The ratio between the upward and vertical, *i.e.*, downward illumination U/V is shown in Table II. The maximum value of 6·9 for the surface waters at Porto Novo occurs at about 590 mμ. It is shifted towards longer wavelengths, the transmission maximum being at about 465 mμ. A maximum value in U/V ranging from 5–7 per cent. at about 425 mμ in the clearest ocean waters has been recorded by Jerlov (1951). He remarks that the ocean is rather poor in scattered long waves in consequence of its low content of particles as compared with the abundance of such in coastal waters.

Further work is in progress with regard to the effect of light on the distribution of planktonic organisms and the amount of radiant energy available at different depths for photosynthetic activity in the estuary and inshore waters.

SUMMARY

1. Measurements of the penetration of daylight of different wavelengths have been carried out in the coastal waters of Porto Novo using photoelectric cells.

2. Nearly 50 per cent. of solar radiation at near ultra-violet region is absorbed by the first one meter of sea-water.

3. Longer wavelengths, especially red light, is heavily absorbed by the surface layer.

4. Selective scattering in the wavelengths 475–590 mμ has been observed in the coastal waters at Porto Novo.

ACKNOWLEDGEMENT

I am thankful to Prof. R. V. Seshaiya, F.A.SC., Director, Marine Biological Station, for suggesting the problem and for guidance.

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STATEMENT OF PROBLEMS AND MEANS OF INCREASING RICE PRODUCTION

By Dr. K. Ramiah

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India has the largest area under rice (75 million acres) among the rice growing countries of the world, and her production, about 25 million tons of white rice, is the second largest. Rice is the most important staple food of the country and contributes to over 45 per cent. of the total cereal production. The average acre yield of about 800 lb. of rice is perhaps the lowest in Asia, the only other country that records still lower yields being the Philippines. India and Japan are the two largest rice deficit countries of Asia. During and immediately after World War II, world rice production was considerably short of requirements, price shot up and rice deficit countries experienced great difficulties.

The chief rice surplus countries of Asia, namely, Burma, Thailand and Indo-China before World War II used to meet not only the deficit of other Asian countries but also export appreciable quantities of rice to markets outside Asia. At the present moment, however, they are not in a position even to meet the complete needs of Asia. Because of the great rice shortage and the consequent more favourable price for rice, countries outside Asia that previously did not grow much rice increased their production, and among these, U.S. is the most important. She has become the third largest rice exporting country now. All countries in Asia took steps to increase their internal production, and it may be said that the pre-war level of production was reached by about 1951–52. Favourable seasons and good harvests in 1953 and 1954 created a temporary situation of falling prices and rice surplus countries meeting difficulties to dispose off their surplus. Among the remedies considered to relieve the situation was one of advising rice deficit countries of South-East Asia that they should not aim at self-sufficiency because the economy of rice surplus countries was so much dependent on their rice exports. This, however, was only a temporary phase and the present position is again what used to exist previous to 1953.

Steps taken to increase internal production has given somewhat satisfactory results in many countries, but the most outstanding results have been obtained in Japan. The increased production has, however, been more than

off-set by the increase in population. The additional production in all countries including India has mostly come from increase in the area under the crop. Japan is the only country where increased production has followed increased acre yields as a result of adopting intensified cultivation practices. It may be mentioned here that generally increase in area is accompanied by a fall in acre yields.

Scope for increasing the area under the crop does not exist in many countries and even where they do, may involve a good deal of capital investment, and the new area may not be quite suitable for the crop. India has to depend more and more upon increasing her acre yields rather than on increasing the area under the crop. A rough examination of the existing statistics over a period of 50 years from 1900 does indicate a definite downward trend in acre yields. This is in contrast with the position in Japan where the acre yields has a marked upward trend though this was not very apparent during the period of World War II. The comparison between the two countries may not be strictly valid because, firstly, the total rice area of Japan is only a small fraction of that of India, and secondly, rice growing conditions in Japan are relatively much more uniform than in the continent of India. A closer study* of the acre yields in India for a 5 year post-plan period does indicate a 10 per cent. improvement corresponding to a 2 per cent. annual increase. This increase is not, however, evenly spread over the whole rice area of the country. The increase has come about by an increase in the acre yields of nearly 16–17 per cent. in the two States of Madras and Andhra Pradesh, about 9 per cent. increase in the yields of Mysore, West Bengal and Madhya Pradesh, and there has been no appreciable improvement in the acre yields of other States. The acre yields of the problem States of East Uttar Pradesh, Bihar and Orissa bring down the average for the whole country. It has to be mentioned in this connection that the present upward trend in higher acre yields, if maintained, can only meet the continuously increasing demand for more food because of the rapidly increasing population. It will not allow for any improvement in the present level of *per capita* cereal consumption which is the lowest, and much below the optimum requirements.

The chief methods available to increase acre yields are: (i) improvement of water facilities, (ii) improvement of varieties by breeding, (iii) improvement of the fertility of the soil, (iv) improvement of cultivation practices and (v) prevention of losses due to pests and diseases. These are briefly referred to in the following pages.

* Information provided by Dr. V. G. Panse, Statistical Adviser, I.C.A.R.

1. With regard to improving water facilities several projects, large and small, have been executed and their role in increasing production is just becoming significant. It has to be understood that only one-third of the rice area in the country has irrigation facilities. Some knowledge exists on the water requirements of the crop, but this knowledge is more empirical and a good deal of research is needed to understand the soil-water-plant relationship. Critical information on the water requirements at the different phases of the plant's growth does not exist. It is known that lack of water at critical stages of plant's growth can appreciably affect production. It can also be said that lack of drainage, characteristic of the lower portions of river deltas, also affects production. A study of the relation between water-supply by irrigation or by rain does indicate that regulated and adequate water-supply by irrigation is much more important than rain.

2. It can be said that India has made a considerable advance in rice breeding, but this advance is not common to the whole country. It needs intensification in several areas. One reason that rice breeding has not contributed tangibly to increased production is the lack of suitable organisation for seed multiplication and distribution of improved varieties. The matter has started receiving attention only in recent years. The principal objective in breeding has hitherto been increased acre yields though other benefits have been obtained incidentally. Breeding for resistance to diseases has also received some attention with noteworthy success in Madras. The present position of varieties calls for intensification of breeding with additional objectives. The most important of these objectives are: (i) varieties with strong non-lodging straw, (ii) varieties with a greater response to soil fertility, (iii) varieties non-sensitive to photoperiod, (iv) varieties that will mature early, etc. The International Rice Hybridisation Project initiated by F.A.O. in 1951 had the above objectives in view. Additional objectives in breeding that offer promise are resistance to adverse factors and improved nutritional quality. The lack of trained personnel and facilities is, however, a bottleneck operating against intensifying breeding programmes in all rice States. Breeding for several of the objectives mentioned above can, however, lend itself to a co-operative undertaking among the States.

3. Ideas on soil fertility have changed and soil survey and chemical analysis of the soil are not the sole criterion for determining fertility. The soil analysis has to be integrated with extensive field experiments. In rice areas where only a single crop of rice is grown in a year, the fallow period helps the soil to recoup fertility, and acre yield is maintained at a constant though low level from year to year. Experimental work has shown that

additions of both organic and inorganic fertilisers to this crop can increase production. One of the easiest methods of supplying organic manure for rice is by growing a green manure crop, often a legume, and turning it into the soil before rice is planted. This is the cheapest form of manuring rice crop and the practice has to be intensified wherever facilities exist or can be managed. A judicious application of chemical fertilisers in addition to organic manures is a sure means of increasing production. Though more research is required there is enough information that has become available in the last few years which can be utilised to go ahead with the use of chemical fertilisers in suitable quantities in different parts of India. Rice growers, particularly in the Southern States, are getting increasingly fertiliser minded, and the present difficulty is lack of adequate supplies to meet the increasing demand.

Among the essential nutrients, the greatest need for rice is nitrogen and the response to application of nitrogen is practically universal throughout the country. The response for phosphorus is somewhat lower than for nitrogen, but the need for phosphorus is found to exist on nearly 50 per cent. of the total rice area in the country. In fact, in some areas as in parts of Assam, Madhya Pradesh and Bihar, phosphorus is the limiting factor to increased rice production. In these areas no response is obtained for nitrogen without the addition of phosphorus. The all-India fertiliser trials in the cultivators' fields have shown that response to nitrogen at 30 lb. per acre is high in all places and the response ranges from 330–886 lb. per acre. The response to an equal quantity of phosphorus is much more variable and ranges from 90–940 lb. per acre. Based on the soil types the response to nitrogen over phosphates is more definite according to the soil type. It is low in coastal alluvium and laterite soils, but high in medium black, red and gravelly soils. These trials have also shown that the response to nitrogen and phosphorus obtained in cultivators' fields are much higher than those obtained in experimental stations in earlier years. Besides nitrogen and phosphorus, special areas have also shown response to potash.

Although ammonium sulphate has been the chief nitrogenous fertiliser in use so far, experiments have shown that other more concentrated nutrients like urea and ammonium nitrate can give equally satisfactory results. It is possible that the efficiency of these fertilisers may be somewhat lower than that of ammonium sulphate in some areas, but the greater concentration of nitrogen and less cost per lb. of nitrogen are points in their favour. With regard to requirements of fertilisers, it has to be understood that organic manures and chemical fertilisers are different in their functions and have

to be looked upon as complementary agents in crop production rather than as a substitute for each other.

Fundamental to the use of fertilisers is the study of chemistry of swamp soils. This has not received much attention in India so far. Work would be particularly desirable to obtain information on the availability or otherwise of particular nutrients as a result of the swamp conditions under which the plant grows. For example, the rice plant needs a considerable amount of silica and iron which the plant can utilise only under swamp conditions. Connected with the use of fertilisers, investigations would also appear necessary to determine how much of the nutrients applied to the soil is actually utilised by the plant. Information is lacking on the question of absorption and assimilation of nutrients by the rice plant at different phases of its growth. It is only such information that can ultimately lead to more dependable results on the question of nutrients to be applied, and how and when they are to be applied to get the maximum benefit.

Experiments at the Cuttack Rice Research Institute and also elsewhere have definitely shown that the application of nitrogen in the form of ammonium sulphate in the subsurface (plough sole) is much more efficient than surface application. There is urgent need for extending this practice under cultivators' conditions. There is also information available that the nitrogen is better applied in split doses at different growth phases of the plant, particularly, when the variety grown takes 150 days or more to reach maturity.

4. Wet rice cultivation does not offer much scope for introduction of large-scale power-driven machinery. There is considerable scope, however, for introduction of improved hand and animal-driven implements. In some areas where carrying out the field operations in proper time is more important, even power-driven machinery may offer scope provided they are either co-operatively owned or can be hired out to growers at reasonable rates. Two operations that may be considered in this connection are preparation of the land for planting, and threshing of the harvested produce. Transplanting seedlings in puddle is the most arduous operation concerned in wet rice cultivation, and research is needed to develop a direct method of sowing the crop, and experimental results already available indicate considerable scope for the practice. One operation that does not receive sufficient attention in India and which may be responsible for a loss of 10–15 per cent. in yield is weeding. More advanced countries where labour is more expensive than in India are successfully using herbicides to control weeds. Their efficiency has also been established in India, but they cost more than hand-weeding.

5. Post-war research on chemical insecticides has produced phenomenal results, and very efficient insecticides are now available. With a suitable organisation to provide timely help to the rice grower, loss of crop due to insects can be almost completely eliminated. Chemicals may also be useful to control some diseases, but the more effective means of preventing losses due to diseases will be to intensify breeding and to evolve disease resistant varieties. Breeding for resistance to blast has proved successful in Madras and since this is the most important disease of the southern region, there is scope for undertaking a co-operative project of breeding for resistance to blast among the States of the region. Breeding for resistance to insects has not received much attention, but the enormous variability present in rice varieties of resistance to stem-borer, the most pernicious insect pest of rice, can be utilised in a suitable breeding programme.

6. In considering various methods of increasing production, it is necessary to emphasise that the greatest benefit can accrue only from a fully co-ordinated programme where all aspects of production receive attention. The importance of this point has become particularly apparent in the programme of the Japanese method of rice cultivation which is being followed in different parts of India. The features connected with the Japanese method are several in number, but the full benefit of the method is realised only when all the features are followed together. It has been estimated by experiments that application of chemical fertilisers is the most important feature of the Japanese method and accounts for nearly 60–70 per cent. of the higher yield obtained as a result of the method. The other cultivation practices associated with the method account for the rest of the higher yield obtained. It should be mentioned that the essential elements of the Japanese method are inter-related and lack of any one of these is likely to reduce the efficiency of others.

BOTANICAL IMPROVEMENT OF VARIETIES— GENERAL CHARACTERS OF INDIAN VARIETIES AND THE APPLICATION OF GENETICS TO RICE IMPROVEMENT

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Abstract

Indian rices belong to the *indica* group of *Oryza sativa* Linn. They are characterised by wide diversity in their morphological and physiological characters. They vary in duration from 85–200 days, in their adaptability to different climatic conditions from cultivation at sea-level to that, at an altitude of 4,000–5,000 feet. They vary in their adaptability to growing in different seasons of the year, and in growing under upland and deep water conditions. They also vary in their resistance to diseases, insect pests and drought. They exhibit wide variation in plant height, tillering, stiffness of straw, grain shedding habit, panicle length, size of grains as well as distribution of pigment on plant parts.

The main problem in rice is the production of high yielding varieties and about 445 improved varieties giving 12–20 per cent. enchanced yield have been evolved in different States by introduction, selection in natural populations and hybridization. Other problems which have received attention at the hands of rice breeders are the evolution of early maturing varieties; varieties responsive to heavy manuring; non-lodging and non-shedding varieties; varieties resistant to floods, salinity, drought, diseases and insect pests, as well as varieties with profuse tillering habit and higher milling out-turn. In all the above breeding projects the principles of heridity, variation and genetic recombination as well as the principle of differential response of genotypes under different environmental conditions, have been fully utilised for the improvement of the rice crop, and several varieties have been evolved or are in the process of evolution, to meet the different requirements.

Introduction

The Indian rices belong to the *indica* group of *Oryza sativa* Linn. In India rice has been intensively cultivated over a long period under conditions favourable for development of local types, with the result that the crop is now a vast complex of different forms and thousands of varieties are found showing wide variation in morphological and physiological characters.

From the botanical survey of the Jeypore tract of Koraput District in Orissa, which is supposed to be a secondary centre of origin of rice, in a period of two years over 1000 distinct varieties have been collected and only a part of the area has so far been covered. Among the physiological characters, two important ones are duration and adaptation to particular seasons. In duration the varieties vary from 85–200 days. Varieties adapted to different seasons are prevalent, and rice is grown throughout the year, in some part or other of India. As regards water requirements, at one extreme there are varieties which are grown under upland conditions, while at the other extreme there are the deep-water rices of Assam and West Bengal, which grow in 15–20 feet of water. Varieties resistant to salinity are found in different States. The cultivation of rice crop extends from sea-level to high altitudes of 4,000–5,000 feet as in Kashmir and different varieties are grown for different altitudes and temperatures. In morphological characters the occurrence and distribution of anthocyanin pigmentation in the plant organs is very variable. The plants range from fully green in all parts to pigmentation present in only one, two or more parts to fully pigmented in all parts. In height, the plant (excluding dwarfs) varies from 2½ feet to 20 feet. In the number of fertile tillers, the variation may be from 2–3 to 25–30 tillers per plant. The panicle size ranges from 13–42 cm. In grain dimensions, length varies from 5·3–11·8 mm. and the breadth from 1·9–3·1 mm. Exceptional panicle types like " cluster " where the spikelets occur in whorls, are cultivated in several regions. In kernel types, red rices are grown in almost all the States and are preferred in Malabar and Kerala. Fine, scented rices are grown all over India and are preferred by a richer section of the population for preparation of special dishes. Glutinous types are grown on a small-scale in all the rice areas for special purposes.

Almost all Indian rices are weak strawed and susceptible to lodging, which brings about considerable loss in yield, ranging from 12–33 per cent. depending upon the stage at which lodging occurs. They are unable to stand heavy manuring and are comparatively low yielders. Most of them are susceptible to diseases like blast (caused by *Piricularia oryzæ*) and insect pests like stem borer (*Schœnobius incertulus* Wlk.), gall-fly (*Pachydiplosis oryzæ*), etc. There is considerable variation in the amount of shattering or shedding in the varieties. A certain amount of shedding is present in most of the varieties; while some are highly shattering others are practically non-shattering. Almost all Indian rices are photo-sensitive and changes in day length cause changes in their growth pattern which ultimately reflects in their yielding ability.

Systematic rice breeding work in India was started about 46 years ago with the appointment of a full time Botanist in the old province of Bengal in 1911. Madras followed suit almost immediately afterwards and since then rice breeding has gradually been taken up in all rice growing States. The Indian Council of Agricultural Research, since its inception in 1929, has encouraged and assisted rice breeding work in the country.

The main problem in India, as in all rice growing countries of the world, is the production of high yielding varieties. Among other problems may be mentioned breeding for earliness, resistance to diseases and pests, resistance to floods, drought, salinity, lodging and shedding of grain, breeding for higher milling, profuse tillering, etc. and the principles of heredity and variation, the principle of genetic recombination of characters from the parents into the offspring, the principle of selection within a heterozygous population and the knowledge of the variable response of a genotype under different sets of environmental conditions, have been utilized in the botanical improvement of the rice crop.

Breeding for Bigger Yield

Yield is the prime consideration in any breeding programme and in the early stages of rice breeding, attention was mainly directed towards improvement in yield of the existing varieties. Spectacular results have been achieved in this direction and a large number (445) of high yielding varieties have been isolated, out of which 394 have been evolved by selection from naturally variable populations, the remaining being evolved by hybridization (Table I). All these varieties are reported to give 12–20 per cent. enhanced yield and many of them possess other valuable characters like earliness, stiff straw, etc.

The improvement so far achieved has been among the most widely cultivated varieties growing in large concentrated tracts, while there are still extensive areas in different States, where the variability in the natural population has not been sufficiently exploited. This enormous variability, resulting in thousands of varieties, each suited to particular conditions of soil, season, rainfall, etc., has led to the low adaptability of rice varieties. Breeding has therefore to be localized and a large number of improved varieties have to be evolved.

Alongside of exploitation of natural population for increasing production, intensive hybridization projects are in progress all over India, for breeding high yielding varieties with improved ancillary characters.

TABLE I

Number of improved varieties evolved by selection and hybridization in each State

	State	Improved varieties evolved by		Total	Yield of unhusked rice in lb. per acre
		Selection	Hybrid-ization		
1.	Assam	23	6	29	1640–3690
2.	Andhra	69	7	76	1500–4300
3.	Bihar	12	2	14	1830–2050
4.	Bombay	44	1	45	1250–3140
5.	Kerala	14	..	14	2000–2800
6.	Madhya Pradesh ..	9	9	18	1240–2552
7.	Madras	103	10	113	1800–5000
8.	Mysore	17	8	25	..
9.	Orissa	31	3	34	1000–4000
10.	Punjab	8	..	8	2000–4000
11.	Uttar Pradesh ..	22	2	24	2000–3000
12.	West Bengal ..	42	3	45	2000–3690

BREEDING FOR EARLINESS

Indian varieties, as already stated, vary in their total duration from 80–200 days. There are a good number of early varieties grown in different States, *e.g.*, *aus* or *ahu* of Bengal, Bihar and Assam, *beali* or *laghu* of Orissa, *kaurs* and *kuruvais* of Madras, *sathi* of Uttar Pradesh, etc. The early maturing varieties are of special significance for tracts with low and uncertain rainfall such as parts of Bihar, Orissa, Bombay, Madras or tracts where the growing season is short due to early setting in of low temperatures as in Kashmir and Kulu valley, as well as for tracts where double cropping of rice is practiced. Early maturing varieties in general are lower yielding but early maturity can be combined with high yield and several such synthesized varieties, such as ASC. 313–11 of Assam, MTU. 15, of Andhra, Chin. 4 and Bankura 4 of West Bengal, Zinya 31 and Patni 6 of Bombay, CO. 20 of Madras, T. 136 of Uttar Pradesh, etc., are available in India,

Since a large part of the rice area in this country is dependent upon rainfall, the work on the breeding of early maturing, high yielding varieties has been intensified in West Bengal, Uttar Pradesh and other States. At the Central Rice Research Institute, two early and high yielding strains, *i.e.*, CR. 1 and CR. 2 have been evolved from crosses between T. 1145 (a high yielding, medium duration variety) and Ch. 2 (a low yielding, early maturing Chinese variety) and also between T. 90 (a high yielding, fine grained, late maturing variety) and T. 1031 (an early maturing and low yielding Chinese variety). CR. 1 (medium coarse) and CR. 2 (superfine) have yielded about 3,200 and 2,300 lb. of grain per acre respectively in a large-scale trial this year. These strains are reported to have done well in West Bengal as well. The performance of some promising cultures (obtained in this project) during 1956–57 is given in Table II.

TABLE II

Performance of some hybrid strains from the early × high yield project at the Central Rice Research Institute in 1956–57

Cross combination	Culture No.	Total duration days	Calculated yield/ acre lb.	% over T. 1145 (control)	% over T. 141 (control)	Grain size
T. 141 ..	Control	144	2444	124·2	100·0	Medium
T. 1145 × Ch. 2 ..	42–76–95–2–1	122	2343	119·1	95·9	do.
T. 1145 × Sathika ..	7–13–43–95	122	2315	117·6	94·7	do.
T. 1145 × Sathika ..	7–6–30–58	125	2283	116·0	93·4	Medium
T. 1242 × Sathika ..	112–241–2–57	130	2210	112·3	90·4	do.
T. 1242 × Sathika ..	112–241–2–68	130	2121	107·8	86·8	do.
T. 1145 × Ch. 2 ..	42–76–92–17	124	2121	107·8	86·8	do.
R. 76 ..	Control	123	2113	107·4	86·5	Coarse, rice red
T. 1145 ..	Control	135	1968	100·0	60·5	Coarse

C.D. at 5% level = 339·2 lb./acre.

Some of the early maturing introductions, especially those from China have shown great promise. They have given outstanding performances under upland conditions in several States, *e.g.*, Ch. 4 in Uttar Pradesh, Ch. 45 in Andhra, Ch. 10, 41, 42, 43, 45, 62, 1007, 1039 and 1040 in Bihar, Ch. 41, 43, 62 and 63 in Bombay, Ch. 45, 47 and 988 in the Punjab. Some of the Chinese introductions from the Central Rice Research Institute appear to be especially suited for high altitudes, *e.g.*, Kashmir and Almora (Uttar Pradesh), where

yields of over 3,000 lb. per acre have been recorded. The performance of some of these varieties is given in Tables III and IV.

TABLE III

Performance of Chinese varieties (sent from the Central Rice Research Institute) over a three-year period, in replicated trials at Vivekananda Laboratory in Almora (U.P.)

	Name of the variety	Source	Cal. yield/acre (in lb.)			Mean yield/acre over 3-year period	Percentage over *Thapachini* (control)
			1955–56	1956–57	1957–58		
1.	*Kaohsiung* 22 ..	China	2016	3544†	5672†	3744	174·4
2.	*Taichung* 65 ..	Formosa	1984	3064†	4600*	3216	149·8
3.	*Sinchu* 50 ..	China	1904	3120†	4152*	3059	142·5
4.	*Chainan* 8 ..	China	1840	3560†	5032*	3477	161·7
5.	*Chainan* 2 ..	China	1296	3264†	4560*	3040	141·6
6.	*Thapachini* (control)	U.P.	..	1912	4528*	2147	100·0
7.	*China* 4 (control)	Acclimatised Chinese type commonly cultivated in U.P.	..	..	3128	..	..
C.D. at 5% level (lb./acre.)				574	547		

* Indicates significantly superior over variety Ch. 4 (Control).
† Indicates significantly superior over variety *Thapachini* (Control).

TABLE IV

Yield data of Chinese varieties from the Central Rice Research Institute tested in Kashmir during 1954–55

	Variety	Source	Calculated yield per acre (lb.)	Percentage over Ch. 1039 (standard)
1.	AC. 1325 ..	China	4485	128·3
2.	AC. 1344 ..	China	3900	111·8
3.	AC. 2992 ..	Taiwan	3900	111·8
4.	AC. 79 ..	Taiwan	3900	111·8
5.	AC. 3 ..	China	3770	108·0
6.	Ch. 1039 ..	Chinese type acclimatised in Kashmir	3490	100·0

Breeding for Response to Heavy Manuring

With the present drive for intensive cultivation to increase production, there is urgent need to produce varieties which would respond to heavy manuring. Indian varieties, as already stated, belong to the *indica* group of *O. sativa* and are in general not responsive to heavy fertilization of the soil. Application of heavy doses of fertilizers induces in them rank vegetative growth resulting in lodging at early stages of plant growth, without significantly increasing grain yield; in many cases yield has been found to be depressed due to heavy doses of fertilizer application. The other group of *O. sativa*, *viz.*, the *japonica* to which belong the rices grown in the sub-tropical and warm temperate regions beyond 30° North and South Latitudes, *e.g.*, Japan, Korea, North China and other sub-tropical countries, is on the other hand composed of varieties which are high yielding and highly responsive to heavy manuring. The *japonica* rices, however, when introduced in the *indica* zone, have in general proved a failure, as the plants are very poor in growth, tillering with very small panicles. This is mainly due to the shorter day-lengths and higher temperatures that prevail under tropical conditions, which cause the *japonica* rices (adapted to longer day-length and lower temperatures) to flower prematurely without putting up sufficient vegetative growth, thus resulting in low yields. Though the *japonicas* were not found suitable for direct introduction, it was thought to utilize them as parents for evolving rice strains combining the *japonica* characteristics of high yield and good response to heavy manuring with adaptation to tropical conditions characteristic of the *indicas*. With this object, two parallel hybridization projects between *japonica* and *indica* rices—sponsored by the F.A.O. of the United Nations and the Indian Council of Agricultural Research for the benefit of South and South-East Asian countries and Indian states respectively—were started at the Central Rice Research Institute in 1950.

In these two projects 192 *indica* varieties selected by the Indian States and the various participating Asian countries were crossed to several *japonica* varieties obtained from Japan and in all 710 *japonica-indica* cross combinations were made. The seed from the hybrids of the various cross combinations was distributed to the States in India and the participating Asian countries, for growing the F_2 and the subsequent generations for further breeding work, under the environmental conditions prevailing in the tracts, where the improved strains were scheduled to be ultimately grown.

The F_1 generation hybrids besides showing marked heterosis particularly in tillering number, showed varying degrees of spikelet sterility ranging from 10 per cent. to almost 90 per cent. This high sterility appeared to be

a bottle-neck in the breeding project, but later studies of this sterility in the F_2 generation showed that the variance and mean sterility percentage of the F_2 generation was of the same order in low (25%), medium (50%) and high (80%) sterility F_1 cross combinations and that equally fertile selections could be obtained from high sterility hybrids as from the more fertile hybrids. This view was confirmed from the study of F_3 and F_4 generations and it was found that by continuous selection for high fertility, good fertile lines could be isolated in later generations.

At the Central Rice Research Institute, selections made in several cross combinations involving Indian *indica* parents are in various stages of breeding. These selections were based on earliness, panicle character, spikelet sterility and yield. All the selections yielded 40–80 gm., per plant and were significantly better than the *indica* parents. In the progeny row trial, the F_4 generation cross combinations *Ginbozzu* × *Indrasail* and *Norin* 8 × *Nangquot*, yielded over 100 per cent. more than the *indica* parents and further the same combinations yielded significantly more under high fertility (80 lb. N + 54 lb. P_2O_5) than under normal fertility (40 lb. N + 18 lb. P_2O_5), the increase being 40 per cent. and 20 per cent. respectively. Good results have been achieved in Andhra, West Bengal, Bombay, Madras, Orissa and other States where selections combining good yield, stiff straw and other desirable characters have been isolated.

BREEDING FOR RESISTANCE TO BLAST DISEASE CAUSED BY *Piricularia Oryzæ*

Of the diseases prevalent on the rice crop, blast disease is the most widespread and of common occurrence. The damage is caused directly by the loss of grains due to neck infection and indirectly by leaf infection. Under conditions favourable for the development of the disease, a loss of 43 per cent. of grain yield has been recorded at Cuttack (*Annual Report, The Central Rice Research Institute*, 1952–53). A total destruction of crop by blast over large areas has been reported in recent years from Andhra, Madras, Kerala, Bombay, Orissa and Kashmir States. Cultivation of resistant varieties appears to be the most usefel way of combating the ravages by this disease. Work on breeding strains resistant to this disease was taken up in Madras as early as 1926 and has since been intensively pursued. With the collaboration of the Mycologist and Rice Breeder several new strains combining resistance to blast and other economic characters have been isolated. Among the new varieties produced are two hybrid strains CO. 25 and CO. 26 from the cross CO. 4 × Adt. 10. These new strains however are very late maturing (165–180 days), coarse and not adapted for cultivation in different parts of

the country. There is need for short duration blast resistant varieties and vigorous programme of hybridization has therefore been taken up in several States like Madras, Andhra and Bombay as well as some other States. In Madras one culture No. 9958/1 from the double cross (Adt. 5 × CO. 15) × (Adt. 17 × CO. 15) is slightly susceptible to the disease and yields 4,110 lb. per acre as against 3,774 lb. of Adt. 17. Several other cultures yielding significantly better than the control have been isolated. In Andhra cultures 5,390, 5,392, 5,352 from crosses between improved varieties and CO. 4 (a highly resistant variety) are being tested at district level, while in Bombay resistant parents like CO. 25, CO. 26 and *Intrasal* are being crossed to the State's high yielding but susceptible varieties.

At the Central Rice Research Institute work in collaboration with the Mycologist is in progress since 1953, to evolve high yielding, early to medium duration and blast resistant varieties. High yielding early to medium duration, resistant to slightly susceptible, fairly homozygous selections from the cross between CO. 13 (high yielding, early, susceptible variety) and CO. 25 (very late and resistant variety) are being tested in bulk trials. Performance of some of these cultures in the F_5 generation tested in Progeny Row trial in 1956–57 is given in Table V.

TABLE V

Cross combination	Culture No.	Mean plot yield in oz.	Calculated yield per acre (lb.)	% over CO. 13	Total duration (days)
1. CO. 13 × CO. 25 ..	556–59	35·7	4050	892	129
	610–94	31·5	3575	788	140
	569–36	27·3	3086	683	136
	559–41	26·8	3040	670	131
	562–31	24·3	2759	608	136
	569–49	24·0	2723	603	139
	562–62	23·0	2614	575	132
	545–36	22·6	2563	565	124
	558–59	22·1	2505	553	131
	558–29	22·0	2496	550	124
2. CO. 13 ..	Standard	4·0	454	100	117

Besides breeding blast resistant varieties by hybridization, over 500 varieties have been tested for their reaction to blast, both under artificial infection at the seedling stage in pots and under natural infection at the seedling and adult stages in the field and so far 14 varieties, *viz.*, BJ. 1, Sm. 6, Sm. 8, Sm. 9, CP. 6, CP. 9, AKP. 8, AKP. 9, S. 67, MTU. 5, S. 624, H. 755, Ch. 55, and PTB. 10 have been found to be resistant and moderately resistant to blast. The resistant nature of these varieties has been confirmed from trials conducted under field conditions at 46 centres in various parts of India. More varieties are being tested each year.

Another important disease of rice is the Helminthosporium, caused by the fungus *Helminthosporium oryzæ*. At the Central Rice Research Institute of the nearly 500 varieties tested, six, *viz.*, Ch. 13, Ch. 45, T. 141, BAM. 10, T. 498-2 A and CO. 20 have been found to be resistant under artificial infection and natural infection in the field, in the seedling and adult stages. A breeding programme to evolve Blast-*cum*-Helminthosporium resistant varieties by crossing BAM. 10 (resistant to Helminthosporium but susceptible to blast) and CO. 25 (resistant to Blast but susceptible to Helminthosporium) is in progress at this Institute.

BREEDING FOR RESISTANCE TO INSECT PESTS

Gall-fly is one of the major pests of rice and is prevalent throughout the monsoon period in all the Indian States. Rice varieties exhibit considerable variation in their resistance to particular insect pests. Investigations carried on at the Central Rice Research Institute on the varietal susceptibility to gall-fly show that in general green, scented or purple pigmented (leaf-sheath purple) varieties show much less incidence of this pest than the green non-scented varieties. In Andhra H.R. 35, an improved variety is reported to be fairly resistant to gall-fly. At the Central Rice Research Institute a hybridization project in co-operation with the Entomologist has been taken up to combine high yield and scented rice of T. 412 with the purple and purple wash leaf blade and leaf-sheath pigment of CP. 12 and CH. 17 respectively.

In Madras State, variety CO. 1 evolved from a natural cross in GEB. 24, is reported to be resistant to the stem-borer (*Schænobius incertulas*), while from Bombay State, *Patni* 6, an improved variety, has been found to be resistant to store moth. More work is needed to test all the available varieties in the country for their susceptibility to various insect pests. Work in this connection is in progress at the Central Rice Research Institute where a number of Genetic stocks are being tested every year for their varietal susceptibility to important pests.

Breeding for Resistance to Lodging

Lodging of rice is a very serious problem affecting rice production in India. Most of the Indian varieties including the improved ones, are weak strawed and susceptible to lodging, more so when heavily manured. With the present drive for intensive cultivation, lodging has assumed increased significance. It has already been pointed out that Indian varieties are generally non-responsive to heavy fertilizer application, which induces in them rank vegetative growth resulting in premature lodging. Preliminary studies made at the Central Rice Research Institute on the extent of loss in yield due to lodging show, that losses upto 34% occur when lodging occurs at preflowering stage, while it is reduced to about 12% when lodging occurs during dough and ripening stages. Thus the problem of lodging in rice is a very serious obstacle in the way of increasing production. Breeding projects are under way in many States to evolve non-lodging high yielding strains. The hybrid *Luchai* × *Gurmatia* × Burma No. 2 evolved in Madhya Pradesh is a non-lodging non-shedding and heavy yielding strain capable of responding to heavy doses of manure.

At the Central Rice Research Institute, work is in progress on the evolution of high yielding, early to medium maturing and non-lodging strains. A large number of genetic stocks are being tested under low-lying and high fertility conditions, for their yield and non-lodging habit. In the preliminary trial conducted last year three non-lodging types, *viz.*, AC. 1951 and W. 113 and W. 130 from Indonesia yielded 2,463, 2,255 and 1,754 lb. per acre respectively which was significantly more than that of T. 141 standard, which yielded 1,182 lb./per acre, the increase being 108·5, 90·5 and 48·5 per cent. respectively over the standard. These varieties are somewhat later than T. 141 by 1–15 days. Hybridization between the non-lodging types and the improved Orissa types has been carried out and promising selections from F_2 and F_3 generations yielding 39·5–51·0 gm. of grain per plant with flowering duration varying between 91–119 days, have been isolated and are under study. Some very promising selections have been obtained from crosses between T. 90 (local) and AC. 517 (a highly non-lodging, poor yielding and early variety from U.P.).

Breeding for Resistance to Drought

Of the 75 million acres under rice, only 20 per cent are under irrigation, while the remaining 60 million acres are dependent upon rainfall. Due to irregular and often scanty rains as in the current year, vast areas are subjected to drought conditions resulting in enormous damage to the crop. The problem of evolving drought-resistant varieties or varieties which are

economical in their use of water, has been receiving the attention of the Rice Breeders in different States.

It has been observed that certain varieties grown under restricted conditions of water-supply thrive better than others and this would show the capacity to resist drought is genotypic and governed by specific genes in the varieties concerned. Most of the drought tolerant varieties however are low yielding and there is therefore strong need to produce medium to high yielding, drought-resistant varieties. As the varieties suitable for drought affected areas have to be of short duration, this factor of early maturity is kept in mind in the breeding programmes. In Madras State, one project on the breeding of varieties resistant to drought has been in operation for a number of years and promising homozygous cultures have been isolated from the cross BAM. 3×TKM. 2, and are being tested. From the crosses between cultivated types like CO. 13, GEB. 24, CO. 1, CO. 2, CO. 5 and the wild varieties belonging to *O. sativa* var. *fatua*, five hybrid cultures have been found to be most promising under Coimbatore conditions. Of the several improved strains evolved by different States, a few of them are reported to be drought-resistant as well.

BREEDING FOR NON-SHEDDING CHARACTER

Shedding or shattering of grain from the rice plant before its harvest, is one of the important factors contributing to loss in yield in rice. There is wide variation in this character in different varieties, but certain varieties which are otherwise desirable become unpopular with the cultivators on account of this shedding habit. The problem of shedding is of special importance at high altitudes, *e.g.*, Kashmir, where the normally non-shedding or slightly shedding rice varieties from the plains, become shedding. Shedding has been found to be a heritable character and the problem of shedding is being tackled by hybridization between appropriate parents.

In Madhya Pradesh, it is proposed to cross some of the improved but shedding varieties of the State with a triple cross hybird strain *Luchai*×*Gurmatia*×Burma No. 2 which is high yielding, non-lodging and non-shedding. In Kashmir State, besides selection in natural populations, crosses are being made between local varieties and the non-shedding Chinese and Japanese varieties to evolve high yielding and non-shedding strains.

Among the high yielding improved varieties, MTU. 7 (Andhra), S. 22 (Assam), PTB. 9, CO. 12 and GEB. 24 from Madras, are reported to be non-shedding also.

Breeding for Resistance to Floods and Submersion in Deep Water

Large areas in Assam, Bihar, Kerala, Orissa, Madras, Western India and Andhra, totalling about 3 million acres get flooded to varying depths of water during the monsoons. The depth of water varies from 2 feet to 16–20 feet (as in Assam) and the duration for which the rice plants may remain submerged, varies from a few days in certain regions, to several days as in some parts of Assam, Madras and Kerala. Besides submersion to varying depths of water, the rice plants in many regions like those of Assam and Bihar, have to contend against the mechanical force of the surging flood waters. Thus the problems of each flooded area are peculiar and these have to be studied before embarking upon breeding programmes.

Varieties differ in their capacity to withstand submersion for a particular length of time and also in their capacity to grow fast with the rise of water and thus keep above the surface. States afflicted with the problem of low-lying areas which are inundated with varying depths of water, or those subjected to heavy annual floods, have evolved by breeding, flood-resistant varieties. Notable among these are the long stemmed strains EB. 1 and EB. 2 of Assam, which can stand 8–15 feet of water and others like Ar. 1, Ar. C. 353–148 and Ar. 614–256 which can stand 2–6 feet of water. Other strains like FR. 13 A and FR. 43 B of Orissa are resistant to floods, while Hybr. 84 of West Bengal can stand submersion up to 6 feet depth. Similarly certain high yielding strains of Madras are capable of standing submersion up to 3–4 feet depth.

Breeding projects, some partly financed by the Indian Council of Agricultural Research for evolving strains suitable for cultivation in flooded areas, are in progress in Andhra, Bombay, West Bengal and Assam and soon a project is proposed to be started in Madras too. Hybridization projects to evolve flood resistant strains, are in progress in a number of States.

Breeding for Resistance to Salinity

Large areas along the sea-coast in the States of Andhra, West Bengal, Bombay and Madras are subject to salt water inundation. Several States have evolved salt tolerant strains, the most notable among these being the SR. 26 B strain of Orissa, which has been found to be doing well in the salt areas of different States. Others, include Chin. 13 of West Bengal, *Kalarata* 1-24 and *Bhurarata* of Bombay, 349 *Jhona* of the Punjab and T. 21 of Uttar Pradesh.

In Andhra State progenies from 13 cross combinations between improved Coimbatore types and four saline resistant varieties, *e.g.*, T. 892, *Kathir*,

Bairuvadlu and SR. 26 B are under study and promising selections have been isolated. In Bombay progenies of crosses between noble sweet land varieties and saline resistant varieties are under study.

BREEDING FOR SEED DORMANCY

In general, short duration varieties in India lack seed dormancy, with the result that under wet weather conditions at the time of harvest, seeds begin to sprout while still on the panicles, causing considerable losses in yield. Seed dormancy therefore is a desirable character in the short duration varieties. In Madras State, seed dormancy has been found to be an inherited character and is being introduced in the short duration varieties by hybridization between the popular short duration varieties like CO. 10, CO. 13, CO. 18, ADT. 3 and MTU. 3 and the exotic short duration varieties from Iraq and Italy which possess seed dormancy.

BREEDING FOR HIGHER MILLING OUTTURN

The ultimate value of a rice variety depends upon its milling percentage, *i.e.*, the percentage of clean rice to paddy grain. Wide variation is known to exist among varieties for this character. In general, coarse rices give a higher milling outturn than the fine ones; thus for an improved variety to be popular among the cultivators, it must possess a higher milling percentage also. No work appears to have been done in this direction; preliminary work on determining the milling percentage of the genetic stocks maintained at the Central Rice Research Institute, has been taken up to determine the extent of variability in rice for this character, with a view to its utilization in future breeding programmes.

UTILIZATION OF MUTATIONS IN BREEDING

There is a very strong positive correlation between the number of ear-bearing tillers per plant and yield. In rice improvement therefore, this character is given the first place and most of the field selections are based primarily on this character. A dwarf mutation called D. 102 with profuse tillering (80–100 ear-bearing tillers per plant) was observed at the Central Rice Research Institute in the variety T. 1222–12, in 1954. The mutant has short panicles, bearing only 15–20 grains per panicle and yields about 15·0 gm. per plant. With a view to transferring the profuse tillering habit of the mutant into some of the cultivated varieties, the mutant was crossed to the original parent as well as to the variety AC. 9. The F_3 performance of some of the selections is given in Table VI.

While none of the segregates could reach or come near to the 80–100 tiller number of the dwarf mutant, some segregates with 20–25 tiller number

TABLE VI

Cross combination	Culture No.	No. of ear-bearing tillers per plant	Single plant yield (gm.)	Percentage over the cultivated parent	
				Ear-bearing tillers per plant	Single plant yield
0. 102-4×AC. 9 ..	R2–14–6–8	25	62·0	192·3	295·2
	R2–35–3–11	24	60·5	184·6	288·1
	R4–18–4–20	23	57·0	176·9	271·4
	R2–21–4–12	25	55·0	192·3	261·9
	R2–35–2–7	22	55·0	169·2	261·9
	R4–18–4–6	25	51·5	192·3	245·2
	R5–3–2–12	20	50·0	153·8	238·1
C. 9 ..	Normal parent	13	21·0	100·0	100·0
0. 102-4×T. 1222–12 ..	7–1–19	23	56·0	191·9	255·7
	17–4–8	21	48·5	175·0	221·5
	25–3–15	22	47·0	183·3	214·6
	15–8–30	23	46·5	191·9	212·3
	15–8–3	21	46·5	175·0	212·3
	15–3–9	26	46·5	216·7	212·3
1222-12 ..	Normal parent	12	21·9	100·0	100·0

appear to be promising, in that, besides the high tiller number, they combine good yield as well.

IMPROVEMENT BY SECONDARY SELECTION

Most of the improvement in rice in India, has been achieved through primary selections in natural populations; and because rice is a self-fertilized crop, it is presumed that there would not be enough genetic variability in these primary selections to permit of profitable secondary selections. However, yield the main objective in breeding, is a complex character of a polygenic nature, dependent sometimes on a very large number of genes, which may not become homozygous even after many generations of selfing. The value of secondary selection in rice breeding has been studied at the Central Rice Research Institute. Twenty-nine established strains, each arising from an original single plant selection were studied for the purpose. Results showed that secondary selection could be profitable in the case of some varieties while not in others. In the variety T. 1222–12 yield was progressively raised by continued selection from 31·4 per cent. in the first year of selection to 40·0 per cent. in the fourth year. This is shown below.

	Yield per plot oz.	Percentage over bulk
T. 1222–12 (bulk) ..	10·94	100·0
T. 1222–12–116 ..	14·37	131·4
T. 1222–12–116–30 ..	14·57	134·1
T. 1222–12–116–30–58 ..	15·16	138·6
T. 1222–12–116–106 ..	15–32	140·0

Similar results were obtained in the case of variety T. 897, where the yield of the selections T. 897–432 and T. 897–457 significantly increased by 13·0 per cent. and 23·6 per cent. respectively over the T. 897 bulk.

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BOTANICAL IMPROVEMENT OF RICE VARIETIES WITH SPECIAL REFERENCE TO CROSSES TO IMPROVE AGRONOMIC CHARACTERS

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Abstract

Notwithstanding the fact that the post-war increase in the world rice acreage and production has exceeded the pre-war level, there is still a considerable world shortage in rice—a cereal that feeds more than half the world's population. This is so because the growth of the rice-eating population is always out-stripping production. Production of rice should, therefore, be stepped up especially by those countries which still depend on imports.

By crossing eight *Indica* varieties of Andhra Pradesh with fourteen suitable *Japonicas*, in forty-four different cross-combinations, highly desirable progenies have been isolated in Andhra Pradesh. They gave up to one hundred and twenty-four per cent. higher yield (than the Andhra Pradesh parents) and are non-lodging and non-shedding. Their milling and cooking qualities are also good. Thus, the new *Indica Japonica* synthetics are likely to be acceptable alike to the farmer, the miller and the consumer.

For effecting further botanical improvement of rice, it is suggested that a very intensive programme of collection of rice varieties in the *Indica* region be pursued and these new collections bred and studied under conditions of high farming and fertilization. It is also suggested that these new improved *Indicas* be crossed with appropriate *Japonicas* for isolating superior hybrids.

It is further suggested that radio isotopes as a source of radiation be employed for getting new and superior germplasm. Unless new germplasm is rapidly built up, both in the *Indica* and *Japonica* groups, the future course of hybridization may not be very fruitful. The present morphology, anatomy and karyology of the rice plant will have to be suitably modified to make it more efficient and useful for future generations.

Following the serious decline in production during the war years, world rice acreage and production have again increased to such an extent that their levels are even somewhat about the pre-war annual average. Nevertheless, the increase in the number of rice-eating people has been so much greater in the meantime that there is still a considerable world shortage in rice.

Production of rice should, therefore, be increased especially by those countries which still depend on imports. Under the warm and humid monsoon climatic conditions that obtain in South and South-East Asia, no other crop can take the place of rice. Increase in production can be achieved by expanding the areas of production and accelerating the yields per unit area. Limited possibilities exist in South-East Asia for expanding the rice acreage, so they cannot provide the solution to the problem. The final solution would, therefore, seem to rest in increasing yields from an unit area. Yield, in its ultimate analysis, may be said to be the resultant of a parallelogram of forces of which the plant and its heredity form one side while the environment is the other one. The environmental factors such as soils, fertilizers, irrigation, drainage, cultural and plant protection methods will be dealt with by the other speakers of this symposium. Hence, the present paper would try to more or less limit itself to the genetic improvement of varieties by various means including hybridization.

Rice breeding has been in progress in Japan, India, Indonesia, Burma, Ceylon, Pnilippines, etc., for nearly half a century. No doubt the intensity of the work varied from country to country, and in the same country from station to station. But one noticeable difference is that while the breeding work in Japan was—at a later stage—done under conditions of high fertilization, the other countries evolved their new varieties with very limited application of fertilizers. The result has been that the present-day improved Japanese varieties are highly responsive to manuring and can transform the plant nutrients into large quantities of grain. On the other hand, the improved varieties of the *Indica* region, numbering nearly 800 are not adapted to high doses of fertilizers. Whenever they are given high doses of fertilizers they put out profuse vegetative growth and the crop lodges even before flowering. With a view to combine the high yielding quality of the round-grained *Japonica* rice with the adaptability of the long-grained *Indica* type to the varying climatic and soil conditions, as well as the cultural practices of the Indian sub-continent and South-East Asia, the International Rice Hybridization Project was launched in 1950. The project is sponsored by the F.A.O. and herein India is participating with eight other rice-growing countries.

In the present note, reference will be restricted to the personal experience of the writer and his colleagues, gained during the last nine consecutive seasons. Eight *Indica* parents, belonging to the former States of Hyderabad and Andhra, were hybridized (at the Central Rice Research Institute, Cuttack) with fourteen *Japonica* parents in forty-four cross-combinations. These

were studied intensively under conditions of high fertilization and farming at Himayet Sagar, Rudroor, Dindi, Samalkota, and Maruteru. So far, no synthetic variety has been released to the farmer. But it is hoped to do so during the next two or three seasons, as the results obtained so far warrant such an action. In the meanwhile, the following broad permissible conclusions are stated:

The progenies showed transgressive segregation for almost all agronomic characters such as yield, plant habit, height, period of flowering, panicle type, grain texture, lodging, shedding, etc.

1. *Yield.*—After all is said and done, yield is the main factor on the basis of which the new synthetic variety will be either retained or rejected. In this context, it is encouraging to note that the performance of the *Indica-Japonica* hybrid population is promising. Yields as high as 224 per cent. over the *Indica* parent (control) were obtained in replicated progeny row tests at Himayet Sagar. It may be mentioned that the *Japonica* parents, Norin 18, Norin 20, Norin 36 and Rikuu 132, proved themselves to be better combiners than Norin 6, Norin 8, Asahi and Gimbozu. This is true, mainly of early maturing progenies at Himayet Sagar. Similar results are reported from the Agricultural Research Station, Samalkota.

2. *Plant habit.*—With regard to plant habit, it may be stated that almost all the *Indica* types that figured in the hybridization except HR. 22 and HR. 35 are either spreading or semi-spreading while the progenies showed spreading, semi-spreading and compact types. There were quite a number of segregates that were very compact and had stiff straw, a few of them being impressive (Fig. 1). Such compact types that combined other desirable characters like suitable period of maturity, height, non-shedding and yield have been retained for further study.

3. *Lodging.*—Segregates that were compact and errect even at the time of harvest were preferred to the semi-lodging and lodging types. In the crosses of Norin 6 and Norin 8 with HR. 19 there were more number of plants that tended to lodge. But it can be said with confidence that some of the types were very conspicuous by their non-lodging character. It is of interest to note that non-lodging character was very pronounced wherein there was Gimbozu blood.

4. *Height.*—New types that are neither as dwarf as the *Japonica* parents (or even less) nor as tall as some of the *Indica* parents (and more) but those that are of just the medium height as is needed under Indian conditions have been retained. The plant height ranged from 45–190 cm. Those that were

retained had a height range of 110–120 cm. In general, it may be stated that the Gimbozu, Norin 18 and Norin 36 parents produced comparatively short statured progenies while Norin 6 and Norin 8 in combination with HR. 19 and Fukoku combined with HR. 35 accounted for very tall segregates.

5. *Period of flowering.*—The period of flowering, in the case of crosses with HR. 19 and HR. 22, ranged mainly from 75–121 days. (The corresponding *Indica* parents flower round about 90 and 100 days respectively.) In the case of crosses with HR. 35 and RDR. 4, the earliest flowering progeny was of 45 days while the latest flowering segregates took nearly 160 days. There was, therefore, ample scope for selecting plants of different durations. Plants of 80–140 days flowering duration were finally preferred to those that are either too early or too late.

6. *Panicle types.*—As regards panicle types, there was a wide variation from the loose drooping type to the dense compact type of panicles. In some of the crosses, of Fukoku × HR. 35, the panicles were as long as 35 cm. The compact type of panicle being in demand was preferred.

7. *Grain.*—Among the grain types, fine-aromatic, fine-non-aromatic, medium and coarse types were all met with. All these different types were selected because they are needed by one class of consumer or the other. The Fukoku and Zuiho in combination with HR. 35 contributed to a large number of plants with fine grain. The same was the case with Rikuu 132 × HR. 19 and also Norin 18 × HR. 19 and Norin 36 × HR. 19. The parent Gimbozu, in general, gave coarse and round grain of the *Japonica* type. The chalky type of grain came in through the parent Asahi and was completely eliminated.

8. *Shedding.*—With regard to shedding also the range was from highly shattering types to extremely non-shedding types. It is no exaggeration to state that in some of the progenies of Rikuu 132, the non-shedding character was so prominent that the rice grain came off from the hull while threshing (Rikuu 132 × HR. 19—F_{10}). Such types are only useful as genetic stocks for further breeding. During selection, the two extremes were avoided, and only types with fair resistance to shedding were retained.

9. *Reaction to Pests and Diseases.*—The wide variation shown by the *Indica-Japonica* hybrids provided sufficient scope for general observations, to be made on the reaction to pests.

(*a*) *Stem borer.*—Stem borer (*Schenobeus incertellus*) attack was high in progenies of the crosses with HR. 22. The parent HR. 22 is itself compa-

ratively susceptible to stem borer. The incidence was also high in crosses of Asahi×HR. 19.

(*b*) *Thrips*.—Differential reaction was also observed in respect of thrips, attack. The attack was high in progenies of crosses with Gimbozu×HR. 22 and Gimbozu×HR. 19.

(*c*) *Blast*.—One of the progenies in the cross Rikuu 132×GEB. 24 was found to be tolerant to blast disease at Agricultural Research Station, Samalkota.

The observations could not be very critical for want of funds and facilities. It is, therefore, desirable that broad-based units are provided for undertaking such investigations.

Summary

In short, it may be stated that new synthetic *Indica-Japonica* hybrids combining three or four useful agronomic characters including yield have been obtained, thus fully justifying the objectives of the original scheme.

Types yielding as high as 224 per cent. over the control (*Indica* parent) were obtained in replicated progeny row tests at Himayet Sagar. The *Japonica* parents, Norin 20, Rikuu 132, Norin 36 and Norin 18 seem to be better combiners as some of their progenies gave higher yields. The Japanese parents, Gimbozu, Asahi, Norin 6 and Norin 8 did not, however, contribute to large number of high yielding progenies.

Follow-up Programme

The *Indica-Japonica* hybridization project was so far co-ordinated and conducted under the auspices of the F.A.O. and the Indian Council of Agricultural Research. The F.A.O., during the year 1956, recommended to the Member-Governments that such interracial hybridization may be undertaken and followed up, to meet the need of the particular area. It is being planned to launch such a programme for the enlarged State of Andhra Pradesh.

Production of new germplasm with the help of radiation.—World genetic stocks of both *Indica* and *Japonica* groups are being maintained at different research centres. It is felt that this blood bank should be further strengthened by creating new forms using radioisotopes as sources of mutagenes.

Experiments on seeds, using different sources of radiation such as X-rays, gamma rays, electrons, thermal neutrons and fast neutrons, have shown that the neutrons as sources of radiation are more efficient since a more uniform effect is produced regardless of the physiological conditions

of the seed. A few very recent examples in some cereal and non-cereal crops would tend to suggest that similar results may also be possible in rice.

Nilson-Ehle, Gustafson and their associates, working in Sweden on cereals, demonstrated that higher yields, stiffer straw and earlier ripening types could be produced by irradiation.

Experiments with oats indicate that while it has not been possible by hybridization methods, to break the linkage between the susceptibility to Victoria Blight (caused by *Helminthosporium sativum* var. *victoræ*) and resistance to Crown Rust (caused by *Puccinia coronata avenæ*) it is possible to do so with radiation, using thermal (slow) neutrons.

Gregory, working at the North Carolina Experiment Station, showed that by X-raying groundnut seed, new types that were superior in yield, better adapted to mechanical harvesting and resistant to a serious leaf-spot disease could be produced. These experiments also demonstrated that in a stable crop like groundnut where the spontaneous natural mutation rate is low and where due to its natural habit of self-polination, new cross-combinations do not naturally arise, radiation had provided about the only feasible way of introducing new germplasm into this rather stable organism and thereby increased the genetic reservoir from which the plant breeder can extract new and desired types.

The leading Swedish workers in the field of radiation genetics are of the view that most agricultural species, even highly bred ones, like barley, wheat or maize are still rather old-fashioned as to morphology, anatomy and karyology, in spite of fifty years or more of intense breeding work. They need to be reconstructed in agreement with the requirements of modern agriculture facilitating mechanisation and an intensive fertilization of the soil, in this way increasing yield and quality but decreasing labour and cost of cultivation.

Similar remarks would be equally applicable to a crop like rice. Considering the fact that this important crop feeds more than half the world's population, it is suggested that the F.A.O. organise work at a suitable world centre or centres.

FIG. 1

BOTANICAL IMPROVEMENT OF RICE WITH SPECIAL REFERENCE TO WIDE CROSSES

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Abstract

Improvement in the seed is a permanent improvement with more lasting effects than any other method of agricultural improvement. The cultivators have taken up improved seed more readily than any other method of improvement. Hitherto the method of selection was followed extensively for the improvement of rice. It has its own limitations particularly with reference to manuring. It is discussed how the *indica* types do not show as much of response as *Japonica* types.

The possibility of improving by hybridization is discussed with reference to some of the inter-varietal and inter-racial crosses done in Orissa.

The work that is now in progress in the breeding of *Japonica* × *indica* hybrids in Orissa is briefly stated. At present there are 27 crosses of different combinations under study in Orissa. Breeding for short and medium height with *indica* type of grain is in progress. The author could get successful combination of the above two characters in the crosses Asahi × T. 812 and Norin 6 × T. 812. Some of the results obtained are presented. It is proposed to study the response of these fixed hybrids to different doses of manuring.

Introduction

Botanical improvement of crops is the permanent improvement of crops and has more lasting effects than any other method. From a recent survey, it is found that the land under Japanese method of cultivation of rice is reverting back to the ordinary method, though every year, new area is coming up. Some of the cultivators who adopted Japanese method of cultivation are reverting back to their old methods. The only improvement that remained is the usage of improved seed in their lands. There are very few lands which after coming once under improved seed have reverted back to the ordinary seed. In fact, in some of the villages where complete saturation with improved seed is achieved, the number of the varieties have become a bye-word for the classification of the land, in Orissa, *viz.*, 141 land for medium class land and 1242 land as low land. It is a common experience at the distribution centres that the cultivators always come up asking if any

new numbers have come up for release. The great scope for improvement through seed keeps an open mind for the cultivators to accept any improved seed without the least hesitation.

IMPROVEMENT BY SELECTION

The number of varieties released by selection in rice is easily the largest in India than in any other crop. This is due to the fact that there is such a wide range of variation in the morphological and physiological characters, that the scope for selection is almost unlimited. In Orissa alone nearly thirty improved varieties are released on this basis. There is a section of Rice Breeders who believe that the possibilities of improving rice by selection is not exhausted due to the presence of a large number of genes. The problem is whether we are getting all the desirable characters and their combinations to evolve a perfect type. Theoretically this may be an impossibility due to the unfavourable association and linkages of some of the quantitative characters.

It is here that the selection sets a limit for further improvement according to the needs of our present day. Our present need is the evolving of such varieties which respond to heavy manuring with conspicuous differences. Ramiah,[7] Sethi[9] reviewing the work on manuring of rice for the last twenty-five years found that there is no significant response above 30 lb. N per acre. In a more recent report of T.C.M. fertilizer trials in India during 1953–54 except in one or two places the response to 40 lb. N was not encouraging as shown below.

TABLE I

Average response for different levels of nitrogen in combination with 40 lb. P_2O_5 grain-yield in maunds per acre

Centre	Control	Response to N lb. per acre			S.E. of response
		20	40	60	
Shimoga	16·02	1·07	0·98	2·57	2·05
Aduthurai	4·31	5·28	4·49	7·81	1·65
Karjat	34·28	4·49	5·96	6·17	6·05
Ponnampet	29·22	8·77	1·69	3·79	6·12
Warangal	27·63	2·20	3·49	10·05	4·27

It is also a general observation that in plots under Japanese method of cultivation, the response is fair in soils of poor fertility while the same is not observed in soils of high fertility. The problem of manuring of rice revolves itself on the quantum of response by rice plant to different doses of various manures. Unless the cultivators get outstanding response to manuring, in rice, as in sugarcane, potato, tobacco and jute, it is very difficult to induce the cultivators to take up heavy manuring of rice on a large-scale, with fertilizers and other manures. Obviously there are some genetic factors affecting this response which are comprehensively studied by Japanese workers.[3]

Improvement by Hybridization and Classification of Rice under Different Eco-Types

On the basis of the widely varying morphological characters,[2] interfertility relationship,[3] and serological studies Kato and others[3] have broadly classified the rice into two racial groups of *Indica* and *Japonica*.[3] The basis of classification by the above workers is given below:—

Character	*Japonica*	*Indica*
1. Shape and colour of the leaf ..	Narrow and deep green	Broad and light green
2. Angle formed by the flag leaf and the stem	Wide	Narrow
3. Shape of grains ..	Broad and thick cross-section markedly roundish	Generally slender and somewhat flat
4. Presence or absence of the awn or its degree when present	Varying from long awned to awnless	A large majority are awnless with short awn
5. Length and amount of hairs on the glume	Hairs not dense and comparatively long	Hairs comparatively thick and short

Later, Matsuo[4] in a study of 1,400 varieties collected all over the world stated that there are three basic plant-types with a combination of 22 characters. These three basic types of plants according to their centres of distribution in the world are designated as *Japonica*, *Javanica* and *Indica*. Some of the more important characters of the three races are given below:

Character	*Japonica*	*Javanica*	*Indica*
1. Type of grain ..	Short	Large	Narrow
2. Shade of the green plant Colour	Deep	Light	Light
3. No. of tillers ..	Many	Few	Many
4. Stem habit ..	Erect	Erect	Spreading
5. No. of panicles ..	Many	Few	Many
6. Shattering ..	Difficult	Difficult	Easy
7. Weight of panicle ..	Heavy	Heavy	Light
8. Length of stem ..	Short	Long	Long
9. Length of panicle ..	Short	Long	Medium
10. Density of panicle ..	Dense	Medium	Medium

Morinaga[5] summed up that the racial differentiation is caused by a large number of genes and small chromosomal mutations subjected to a selection of different environment.

Great significance is attached to the above classification on account of the relative response to heavy manuring and the consequential yield as achieved in Japan. Matsuo[4] studied the genetical basis of response to nitrogenous fertilizers in the three racial varieties as analysed on the basis of the following characters:—

(1) Power of absorbing and assimilating N element;

(2) Morphological or histological changes arising out of the absorption of N;

(3) Degree of development of lodging;

(4) Incidence of diseases and pests as a result of nitrogenous manuring.

As a result of his study, he has come to certain general conclusions as stated below in the response of the three racial varieties to manuring with nitrogenous fertilizers.

(1) *Japonica* racial varieties are suitable to heavy manuring and thin planting and the *Indica* racial varieties for light manuring and dense planting.

Further, the *Indica* varieties showed the following characteristics:—

(1) Low percentage of nitrogen on dry matter basis during tillering phase;

(2) The period during which the leaves maintained the assimilative power for nitrogen after flowering is very short;

(3) Most of them lodge by heavy manuring;

(4) *Japonica* varieties contain less nitrogen per unit area of fresh leaf than those of *Indica* varieties, while on the whole they have higher percentage of 'nitrogen content on dry matter basis'.

(5) *Japonica* varieties favourably absorb and assimilate nitrogen even under heavy manuring conditions increasing their yield of grain, whereas the *Indica* types absorb much nitrogen during the initial period of its growth as its assimilation does not keep pace with absorption, with the result that the ratio of non-protein nitrogen to the nitrogen is quite higher thus preventing the further absorption of nitrogen.

The net result of the above findings is that the N content of the *Indica* varieties is found to be the same in the later period of growth whether manured or not, and consequently the yield of grains is not remarkable. Therefore it is contended that the *Japonica* rices show a big difference between the manured and unmanured plots.

These eco-types are generally classified as panicle-weight, and panicle-number types which are relatively adopted to different grades of soil fertility and manuring. According to H. I. Oka[6] the rate of increase in the height of the plant at the time of tillering was greater in *Indica* varieties than in *Japonica* where the number of tillers increased making contribution of a better factor towards yield.

With the above limitations of *Indica* varieties the rice breeders in India have practically come to a blind-end with regard to manuring of rice. It is at this stage thanks to K. Ramiah whose imagination and initiative has opened fresh possibilities of breeding better responding varieties to heavy manuring by the *Japonica* × *Indica* hybridization scheme under the auspices of the F.A.O.

Improvement by Hybridization

Large number of improved varieties are evolved by hybridization also in India. There has been always a specific purpose in the hybridization programme to combine some specific character. Inter-varietal crosses are very common for this purpose. In Orissa BAM. 11 a hybrid variety was evolved

as a result of the cross between Boroponko × Benibhog to combine white kernel colour of Benibhog with the other desirable qualities of Beroponko. During the last ten years this hybrid became very popular in Orissa. There are thousands of acres under this variety now. More recently another hybrid is released as Hybrid 5, a cross between a Punjab variety P. 10–6 and a Hyderabad variety H.R. 19. This hybrid which is suited for high lands with a second crop programme is under multiplication in different seed farms. Interspecific crosses also were made between a popular variety BAM 3 and *O. perennis*. After following up the segregation, twelve drought-resistant selections were made. In 1955 further crosses were made between T. 141 × T. 442 and BAM. 3 × FRNS. 1740 (an all-purple type) to combine the purple leaf-sheath with the popular strains of T. 141 and BAM 3. At present studies in F-3 generation are in progress.

WIDE CROSSES

Till the F.A.O. project of *Japonica* × *Indica* hybrids was started in the C.R.R.I. no serious attempts were made for the inter-cross-breeding of phylogenetically distinct groups of rice. According to Morinaga and Fullushima[5] hybrids of these widely different groups are highly sterile due to the bringing together of the unbalanced groups of genes. Even when the fertile hybrids are produced the progenies are extremely heterogeneous segregating into endless variation of characters, due to the complicated lineage of *Japonica* parents. In a number of crosses made in Japan with the Indian varieties fertility upto 80% was obtained (Morinaga[5]). Under the F.A.O. project F-2 seeds of the following crosses were sent to the Agricultural Department of Orissa.

In the earlier cross *Indica* types were made the maternal parents and in the latter crosses the position was reversed.

Crosses with Indica *Types as Maternal Parents*

T. 1145 ×	Asahi Norin I Aikoku	3 crosses
T. 90 ×	Rikku 132 Thaichu 65	2 crosses
T. 1242 ×	Rikku 132	1 cross
BAM. 9 ×	Aikoku Asahi	2 crosses
812 ×	Rikku 132	1 cross
	TOTAL	9 crosses

Crosses with Japonica *Types as Maternal Parents*

Taichu 65
Norin 6
Norin 8
Norin 18
Norin 20
Rikku 132
Asahi
Gimboju
× T. 1145 = 8 crosses
× T. 812 = 8 crosses

Norin 18 × T. 90

Norin 8 × T. 1242

All the above crosses at different stages of purity are under study.

Sterility

The range of fertility was only 15–30% in all the crosses. Within this range there was variation within the crosses. High grades of fertility were found in Asahi×812 and Norin 6×T. 812. Completely fertile hybrids were obtained in F-4. As reported by H. I. Oka[6] the fertility in wide crosses is controlled by polygenes which are responsible for gamete development sets of duplicate genes. If both are recessive they cause deterioration.

Breeding for Short-Height and Heavy Tillering

Height.—Height as a quantitative character is greatly conditioned by the environmental factors. It is an important factor which considerably affects the response to heavy manuring. The general height in *Indica* types varies from 130–200 cm. The average height of the *Indica* parents selected for these crosses is about 160 cm., and are very susceptible to lodging under heavy manuring conditions. Both monogenic and polygenic segregation was reported by the previous workers. In the present investigation plants with short height are always found recessive. Further, it has been found that the recombination of genes for the desirable characters is very much restricted in the phylogenetically distant types. As it is stated by Oka[6] the interaction of the genes regulating the height, panicle number and grain size is very significant. The segregating population was divided into three classes according to their range of height, all the plants below 100 cm. height as short, 100–120 cm. as medium and above 120 cm. as tall. Majority of short segregates were early and being recessives they were scarce. They were smothered by the tall plants. Comparatively the incidence of short plants in medium and late groups is very low. The statement below shows the frequency of the plants in different classes under different groups.[1]

Name of research station	Height up to 100 cm.			Height up to 101–120 cm.			Above 121 cm.		
	Early	Medium	Late	Early	Medium	Late	Early	Medium	Late
	1	2	3	1	2	3	1	2	3
Jeypore ..	345	65	4	299	125	10	91	65	2
Bhubaneswar ..	59	19	4	153	208	25	170	276	86
Berhampur ..	7	Nil	Nil	48	40	12	172	418	303
TOTAL ..	411	84	8	500	373	47	433	759	391

It has been observed by many workers that wherever the short plants were obtained, in majority of the plants the nature of grain was invariably that of *Japonica*. It is a rare combination to get short plant with *Indica* type of grain particularly of the superfine quality. The author considers himself fortunate that he could get such combinations in two crosses of Asahi×T. 812 and Norin 6×T. 812. Now there are a number of cultures under F-7 which are fairly fixed for all the other morphological characters. Being short in height they are non-lodging though they are grown under 60 lb. N. All these short cultures are invariably associated with narrow dark green leaf. During the current season also, the number of cultures which are breeding true to their class in height are given in the statement on next page.

Ramiah *et al.*[8] found that the short-round grain which represents the *Japonica* type is dominant over the long medium grain of *Indica* types, and according to Bhide (1926), Majid (1939) and Chakravarty (1939) the grain size is controlled by multiple genes. With two important characters as recessives both of which are controlled by polygenes the chances of getting a double recessive are very limited for which a very large segregating population is required.

TILLERS

In almost all the cases short-height is associated with good tillering and short ears. Until further detailed studies are made into their response to heavy manuring and other cultural methods, their contribution to yield cannot be assessed. As the situation stands, plants of medium height with the range between 100–120 cm. are better contributors to yield under our present conditions of manuring.

Cross	Below 100 cm. height	101–120 cm.	Above 121 cm.
Norin 6×T. 812 ..	35	2	..
Norin 20×T. 812 ..	8	4	2
Asahi×T. 812 ..	17	16	2
Gimbozu×T. 812 ..	2	4	2
Norin 20×T. 1145 ..	9	..	..
Norin 6×T. 1145 ..	4	..	..

In these medium-sized plants the length of the panicle also is moderately long which is a factor to be reckoned with as far as the cultivator is concerned. As reported earlier by the author a large number of selections was made on the basis of the yield of single plants wherein the selection had to be confined in some of the cultures where the average yield was 50 gm. per plant.

Conclusion

Botanical improvement of rice through wide crosses is considered possible. It has been possible to combine some of the morphological and quantitative characters which are conducive for response to heavy manuring at the same time retaining the quality of the grain of *Indica* types. The crosses Asahi×812 and Norin 6×812 have been particularly favourable for this purpose.

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PRESENT POSITION OF FERTILIZER EXPERIMENTS IN RICE IN INDIA

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Abstract

A large number of fertilizer experiments have been conducted in the country with an object to get a rationalised and economic programme of fertilizer for different areas. Results have shown that rice soils in India need both nitrogen and phosphate for increasing production. Response to potash application has however not been adequate enough. Nitrogenous fertilizers have been found most effective in increasing yield in almost all trials, but highly significant yield increases to phosphate application have been obtained only in a few localised areas in the country. In recent all-India trials conducted on cultivators' fields, however, phosphate need is felt at many places. Green manuring, another rich source of nitrogen, has proved equally effective and sometimes better than inorganic fertilizers. Different nitrogenous fertilizers like ammonium sulphate, ammonium nitrate, ammonium chloride and urea have also been tested. At experimental stations, ammonium sulphate has generally proved more effective than urea and ammonium nitrate but in all-India T.C.M. trials on cultivators' fields, results have been rather variable and not much difference between these sources of nitrogen carriers has been observed. Various phosphatic fertilizers tried are also not found different in their behaviour.

Time of fertilizer application, level and type of fertilizer and magnitude of response is found to vary widely from place to place. No relationship between the soil type and any of these factors has as yet been established. Available soil analytical data have also not thrown light on this aspect. In the absence of this, it becomes difficult to formulate fertilizer schedules and advisory standards for different areas. Apart from soil type, soil texture, drainage and soil water status in rice soils during the growth period may be considered responsible for such wide variation in fertilizer response. Great losses of nitrogen by leaching and denitrification occur when a rice soil profile left in aerated condition for a few days is submerged. Subsurface placement of ammoniacal fertilizer has been suggested as a rational method to reduce such fertilizer losses and increase fertilizer efficiency. Other examples of influence of soil water on fertilizer response by ammonium sulphate and urea are given and urea is considered

more profitable, when used mixed with dry soil and flooded about 48 hours after application.

Physiological acid action of ammonium sulphate is not likely to play any important part in deterioration of soil fertility in water-logged soils, except under highly acid soil conditions. This is because of acid tolerance of rice crop and increase in pH of rice soil on flooding. Danger from ammonium sulphate may however arise due to sulphide injury in soils lacking in active iron and manganese.

Importance of soil-water relationship in fertilizer application in manurial trials is stressed and collection of data on soil water condition before and after fertilizer application and during critical growth condition of the crop up to flowering is suggested so that experiments under similar soil water patterns can be pooled for interpretation of fertilizer response. Separate investigations to study interaction of different soil water patterns with fertilizer response are also suggested. This may give useful information on which fertilizer schedules and advisory standards for different areas may latter be based.

CORRECT use of fertilizer has been recognised as a sure and quick method of increasing rice yields. A large number of experimental trials have been planned on the subject in the country during the last 25–30 years and early work on these has been reviewed by Sethi (1940) and later by Sethi, Ramiah and Abraham (1952). Most of these early trials were conducted on experimental stations which were comparatively few and also had a higher soil fertility status and better soil management; the results of the trial therefore served limited purpose in extending fertilizer practices amongst cultivators. Stewart (1947) therefore suggested carrying out large number of simple and complex trials in cultivators' fields under different soil and climatic conditions to get a rationalised and economic fertilizer programme for different soil types in the country. State of Bihar, and to a limited extent, some other States took advantage of this suggestion and later, from 1953–54 onwards, an all-India programme of manurial trials was started by the Indian Council of Agricultural Research under the auspices of Indo-U.S.A. Technical Co-operation Mission (1955, 1956 and 1957). In addition, a large number of simple and complex trials have also been conducted at State experimental stations and under the Japanese method of cultivation sponsored by the Government.

Results of these vast majority of fertilizer trials conducted in the country have shown that rice soils in India respond both to nitrogen and phosphate fertilization, but response to potash fertilization is not adequate. Table I

shows average nitrogen and phosphate responses in some of the recently conducted all-India T.C.M. and Bihar trials.

TABLE I

Average response in lb. per acre to nitrogen and phosphate application

Experiment	20 lb. N	40 lb. N	20 lb. P_2O_5	40 lb. P_2O_5	20 lb. N+ 40 lb. P_2O_5	40 lb. N+ 40 lb. P_2O_5
T.C.M. trials (average of three years 1953–56)						
Complex trials ..	224	362	164	246	286	353
					20 lb. N+ 20 lb. P_2O_5	
Cultivators' field trials	338	..	271	..	502	..
Bihar State trials (average of two years 1953–55)						
	25 lb. N	50 lb. N				
Cultivators' field trials	414	582	256	355	..	..

In all trials conducted in the country, nitrogen has given the highest yield response, followed by phosphate and generally there has been no evidence of interaction between the two fertilizers. In T.C.M. trials, response to phosphate application has been particularly marked in medium black soils at Bhagwai (M.P.) and red loams at Maruteru (Madras). Instances of nitrogen and phosphate interaction have been reported by Desai and Krishnamoorthy (1954) on certain black soils near Hyderabad (Table II) where, with continuous application of both nitrogen and phosphate fertilizers, yield in some trials has been increased from 440 lb. in 1949 to 6129 lb. per acre in 1953.

TABLE II

Response in lb. per acre to nitrogen and phosphate on black soils near Hyderabad-Dn.

Control yield lb./acre	Yield increase in lb. per acre		
	45 lb. N	45 lb. P_2O_5	45 lb. N+ 45 lb. P_2O_5
655	530	494	2897

Trials on combination of organic and inorganic fertilizers have also been conducted and results obtained have shown that response to nitrogen and phosphate application is generally higher in absence of organic matter. Long-term experiments on combination of bulky organic manures and inorganic nitrogenous fertilizers, conducted at Chinsurah (West Bengal), Cuttack (Orissa), Lambhadi (M.P) and Kudwani (Kashmir) to study the effect on build up of soil fertility have shown that, generally response to application of ammonium sulphate over a basal dressing of Farm-Yard Manure is not superior to application of ammonium sulphate alone. Green manure, another rich source of nitrogen, has been tried in large-scale experimental trials and has proved equally efficacious and sometimes even superior to ammonium sulphate, compared at equal nitrogen basis. Green manure has an advantage, in that it gets thoroughly mixed in the furrow slice at the initial stages, it works as quick acting fertilizer on decomposition of tender and green leaves, later it has a delayed fertilizing action, when the hard woody material starts decomposing. Application of phosphate fertilizer with green manure crops has also proved effective at many places. Phosphate applied to green manure crop, apart from its direct fertility value to rice crop, increases the tonnage of green manure per acre, thus making increased nitrogen available for crop utilization. Under highly impeded drainage conditions, however, large dressings of green manure can have deleterious effect and retard initial crop growth due to accumulation of toxic substances, which act as respiration inhibitors.

Fertilizer experiments on time and method of application have also been tried on a wide variety of soil climatic conditions. Results on time of fertilizer application have varied from place to place and at some places, full dose applied at planting is considered adequate and at other places, split application is found profitable. Results of recently conducted T.C.M. trials have shown that best time of fertilizer application, even for a given centre is found to vary in two years. The time of fertilizer application depends not only on growth period of the crop but also on various other factors such as soil texture, drainage and soil water conditions. Generally open-textured and freely drained soils may need split application of fertilizer, while in lowland and heavy clay soils with impeded drainage, one single application, basal or post-planting may be adequate. In this connection, it is particularly necessary to develop a technique by which fertilizer needs of a growing crop may be easily ascertained and application done accordingly. Mention may be made of one such technique developed by Japanese workers (Ozaki, 1955), who consider absence or presence of asparagine in undeveloped flag leaf before flowering, as need or otherwise for top dressing of crop.

Methods of application to increase fertilizer efficiency have generally not received adequate attention in our fertilizer trials. Ramiah, Vachhani and Abichandani (1951) had suggested subsurface placement of nitrogenous fertilizers as the most effective method of increasing fertilizer efficiency. This method has been tried on an extensive scale at the Central Rice Research Institute, Cuttack, and is found to increase fertilizer efficiency by 1·5–2·0 fold over customary surface application. Various methods of subsurface fertilizer placement have been suggested by Abichandani (1955) to suit different cultivation practices.

Of late, efficiency of a number of new nitrogenous fertilizers in increasing rice yields is being tested in several trials. It has been found at experimental stations that generally ammonium sulphate, ammonium sulphate nitrate and ammonium chloride have almost the same fertility value, while urea, ammonium nitrate and calcium cyanamide have been found inferior (*Rice in India*, 1956). In recently conducted T.C.M. trials, however, fertilizer response to ammonium sulphate, ammonium nitrate and urea was found to vary considerably from place to place and even from year to year at the same place, as such, no definite conclusions have been drawn regarding the superiority of one over the other.

It has been seen that although in all trials response to nitrogen application has been fairly uniform, the level of nitrogen, time of application, type of fertilizer and magnitude of response is found to vary from place to place and also sometimes from year to year at the same place. Because of this, it becomes difficult to set any rationalised fertilizer schedule and advisory standards for different areas, particularly when the results of trials are not co-related with soil type and other soil analytical data. Recently attempts are being made to co-relate responses with soil types but no known relationship has yet been established. Bihar cultivators' field trials, although originally laid out on politically demarcated boundaries (district-wise), were later grouped under alluvial, gneissic and other soil types and also under irrigated and unirrigated conditions. These comparisons have not given any evidence regarding influence of soil type but there was a general tendency for response to be low on lighter soil in any group. A slightly higher response to nitrogen was also obtained under irrigated conditions than under unirrigated conditions. All-India T.C.M. trials were planned under diverse soil types such as black soils, recent alluvium, red soils and lateritic soils, etc., but no definite conclusions regarding influence of soil type on fertilizer action have been arrived at. Soil analytical data for these experiments is not as yet available and some of the differences may be explainable on inherent soil fertility of different

locations. The inherent soil fertility under rice soil condition is however, very intimately connected with soil moisture status during the growth period of the crop, therefore, apart from soil type, the influence of soil moisture conditions at different phases of crop growth may greatly modify the fertilizer action and some of the differences may well be explained on this variation in cultivation conditions.

Most of the rice areas in the country are rainfed, where moisture conditions vary widely and the crop is grown dry, semi-dry or wet depending on the location of the land and intensity and duration of the monsoon. This diverse pattern of the soil moisture conditions not only affects our rice yields but may also influence the fertilizer responses, particularly of nitrogen. In dry or semi-dry aerated state, predominant form of available nitrogen is nitrate, which on flooding and submergence for continued period can suffer loss by leaching and denitrification in subsurface zone of the flooded soil and lose its fertility value. Ammoniacal nitrogen is however stable if applied under submerged soil condition. Thus, alternate drying and wetting of soil under rainfed or irrigated conditions, particularly after fertilizer application can, under adverse conditions, result in considerable losses of applied nitrogen. Under freely drained soil conditions also, these fertilizer losses can be fairly heavy due to leaching and may vary with the type of fertilizer used. Recently some manurial trials on fertilizer soil water response have been conducted at the Central Rice Research Institute, Cuttack. In one series of trials, fertilizer response has been studied with respect to drainage conditions. A series of pots were allowed free drainage, but plants were not allowed to suffer because of lack of water. Another series of pots were not allowed any drainage. These two conditions more or less corresponded to upland freely drained condition and lowland submerged condition respectively, under the same soil type. Yield response with application of ammonium sulphate and urea under these two conditions is given in Table III.

Results show that fertilizer response is generally better under submerged soil condition. Ammonium sulphate and urea are found to have almost the same fertility value under submerged soil state; urea even doing better at lower nitrogen supply. Under freely drained state, however, urea fares very badly compared to ammonium sulphate. Green manure at high nitrogen supply is also found inferior to ammonium sulphate under both conditions. This can be attributed to accumulation of toxic substances in soil which retarded early growth of crop in these treatments.

Another trial on fertilizer soil-water relationship was conducted to study fertilizer response on light and heavy textured soils of the same soil type and

cultivated under similar soil-water relationship. Total nitrogen in soil used for study and no nitrogen yield are given in Table IV and yield response with increasing levels of nitrogen applied as ammonium sulphate is shown graphically in Fig. 1.

TABLE III

Influence of drainge on fertilizer response on clay loam soil (Second season crop)

Drainage condition	Control yield gm./pot	Yield increase in gm. per pot					
		1·0 gm. N			2·0 gm. N		
		Amm. sulph.	Urea	Green manure	Amm. sulph.	Urea	Green manure
Freely drained ..	18	27	13	25	54	8	22
Submerged condition	23	40	58	38	79	72	44

TABLE IV

Total soil nitrogen and no nitrogen yield of grain in heavy and light textured soils (Mahanadi alluvium)

Soil texture	Total N % in soil	No nitrogen yield gm./pot
Heavy clay ..	0·09	33
Clay loam ..	0·08	41
Sandy loam ..	0·06	24

It is seen that soil texture and initial soil fertility both have great modifying influence on fertilizer response. Whereas under clay and clay loam soil, lower level of nitrogen seems to be adequate for optimum yield, under sandy loam soil, a higher nitrogen level is necessary. This experiment has been done under submerged soil conditions with respect to all textural groups; under actual field condition, however, these groups occur under different soil moisture conditions, sandy loams usually having free drainage, the fertilizer responses are therefore likely to differ more widely under actual cultivation conditions.

Question of superiority of ammoniacal and nitrate fertilizers for rice crop has of late assumed importance, particularly because of Government programme to instal suitable fertilizer factories during the Second Five-Year Plan. While the superiority of ammonium sulphate over ammonium

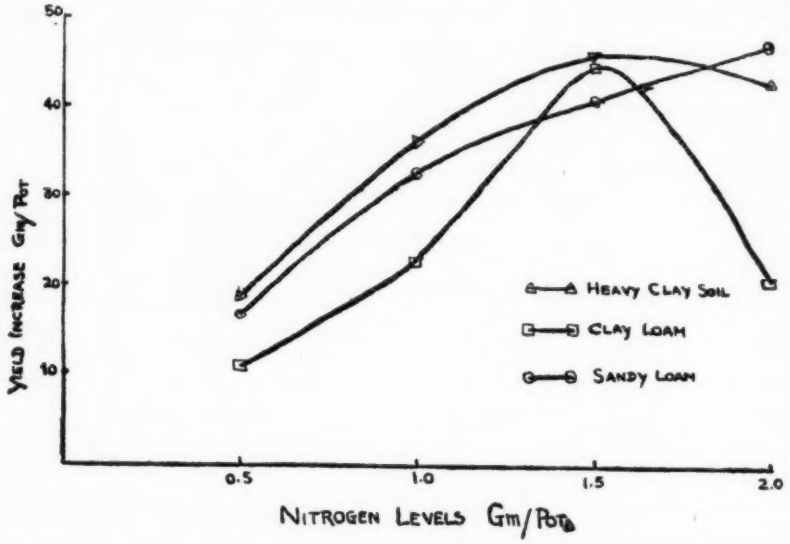

FIG. 1. Response due to nitrogen application in heavy and light textured soils (Mahanadi alluvium)

nitrate has been definitely established from fertilizer experiments conducted at experimental stations (*C.R.R.I. Annual Reports*, 1950–56), evidence from all-India T.C.M. trials has not shown any difference between the two. The fertility value of these two fertilizers is also connected with soil water system under which the fertilizers are used. Experimental evidence collected at the Central Rice Research Institute, Cuttack (unpublished records) on availability of nitrogen from these two fertilizers under rice soil conditions has shown that under continuous wet land condition, ammonium sulphate gives higher nitrogen availability than ammonium nitrate, while under semi-dry and dry land condition of freely drained and intermittently irrigated or flooded soils, both fertilizers behave more or less similarly. Application of urea with respect to soil water conditions has also been examined at the Central Rice Research Institute, Cuttack. It is seen that to increase fertilizer efficiency of urea, it should be applied to soil well mixed in dry or semi-dry state and soil flooded about 48 hours after incorporation. Application of urea on heavily flooded field may not be desirable because of leaching and

surface run-off losses that are likely to occur before the fertilizer is converted to ammoniacal nitrogen form and fixed in the soil complex. Urea may therefore prove more effective under irrigated condition than under lowland rainfed condition.

As regards phosphate fertilizers, various fertilizer trials conducted of late have shown the need for phosphate in increasing crop yields. Response to phosphate application is at present rather low, but with intensive crop production methods and removal of more crop from soil year after year, more need for phosphate application may arise. Phosphate has generally given very good response in certain localised areas of Andhra Pradesh, parts of Bihar and Bombay State. Amongst the different phosphatic fertilizers tried in various trials, generally not much difference between forms of phosphate carriers has been obtained. In T.C.M. trials, superphosphate tried on calcareous and acid soils does not seem to behave very differently. These trials are rather few to warrant any definite conclusions. These all-India trials on cultivators' fields have also not shown any significant difference between superphosphate and bone-meal. Placement of phosphatic fertilizers has been tried at certain places and found superior to broadcast application.

Phosphate fertilization of rice has also to be viewed with respect to soil conditions on flooding. Rice soils, in general, on flooding, contain more soluble phosphate than the same soil under upland aerated condition (Mitsui, 1955 and *C.R.R.I. Annual Report*, 1955–56), as such, application of phosphate at early stage of growth, when the subsoil is not in such a reduced state, is considered as more satisfactory time of application. On this assumption, phosphate application should prove more responsive under upland condition than under flooded condition. No experiment on this aspect with rice crop is however reported in our country. Mention may here be made of contact solution hypothesis of nutrient uptake (Mitsui, 1955). As a practical application of this, it is suggested that water-insoluble phosphatic fertilizer may be well mixed in soil, while the water-soluble phosphate like superphosphate may profitably be applied by placement in order to avoid its complete mixing with the soil over the whole area.

Experiments on potash fertilization of rice have not been very encouraging as the response has been rather low. Mukherjee (1955) from Bihar has reported an average yield increase of 205 lb. of paddy per acre with application of 20–40 lb. K_2O per acre. In T.C.M. trials also response has been generally low, about 80–150 lb. per acre except on certain coastal alluvium, laterite soils and red loams. Examination of irrigation waters of the rivers may be necessary in connection with potash fertilization, as some river waters

may bring ample supplies of potash in irrigation waters. In Japan, potash application has been found generally ineffective in irrigated areas, because of potash deposited in irrigation waters, while in entirely rainfed areas, need for potash application is felt.

Reference may also be made to some liming trials conducted in the country. In some permanent manurial trials laid out at Chinsurah (West Bengal) and at the Central Rice Research Institute, Cuttack (Orissa), lime has been used in trials with an object to correct any physiologically acid action arising out of continued use of ammonium sulphate and levels of quarter to half ton of lime per acre, are applied every alternate year for the last 8–10 years. No advantage due to lime application has so far been reported in these trials (Digar, 1956 and *C.R.R.I. Annual Reports*, 1950–56) during this period. Examination of soils at both places have also not shown any change in soil reaction or exchangeable lime status of the soil in no lime plots, due to continuous use of ammonium sulphate. It may be mentioned that lime, as a corrective of possible acidity due to continuous use of ammonium sulphate, may not prove much effective on submerged rice soils, unless the soil reaction itself is highly acidic and soil is very low in base status, because rice crop is acid tolerant and very good yields have been obtained on acid soils. Besides, during flooding, the pH of rice soil increases because of increase in exchange acidity brought about by reduced condition of the subsoil. Ferric iron in the rice soil gets reduced to ferrous state, a stronger base and sulphate gets reduced to sulphide, a weaker acid. Danger from use of ammonium sulphate may however arise because of sulphide injury in soils lacking in sufficient active iron and manganese. Of late, in liming trials, higher doses of lime ranging from 1–2 tons per acre have been used in T.C.M. trials conducted on acid soils and yield increases ranging from about 350–500 lb. per acre of grain have been obtained. Similar trials were earlier conducted from 1952–53 onwards at the Central Rice Research Institute, Cuttack (*C.R.R.I. Annual Reports*, 1952–53 and 1954–55), where lime applied at about one ton per acre on a soil of pH 6·2 resulted in an yield increase of 350–450 lb. per acre. Investigations have shown that this action of lime is due to its mineralising action on soil nitrogen, releasing additional nitrogen for crop utilization (Mitsui, 1955 and *C.R.R.I.* unpublished records). At the Central Rice Research Institute, Cuttack, one ton of lime applied at puddling has been found equivalent to 30 lb. nitrogen per acre used as ammonium sulphate. Mineralising action of lime on soil nitrogen in submerged soils is connected with increase in initial pH of soil above 8·5 for a short period, which renders some soil organic matter dispersable and

thus easily decomposed by soil micro-organism. This method of releasing additional nitrogen for crop will however depend on the cost of lime and may work out expensive. Besides, it will also be at the expense of initial soil-fertility which may soon get exhausted with repeated lime applications.

The importance of fertilizer use in manurial trials with respect to soil-water relationship is therefore a great contributing factor in determining the success or otherwise of fertilizer application. Fertilizer trials so far conducted in the country have served a very useful purpose in demonstrating fertilizer use in crop production on various soil types. Still greater service can be rendered if in future experimental trials attention is paid to soil-water relationship and data regarding method and time of application of fertilizer and soil water conditions, particularly after fertilizer application and also during the critical growth periods of the crop are kept to enable grouping of data on known soil types and similar soil water patterns. This, along with soil analytical data, will clear some of the diversities of responses obtained under different soil types. Besides this, it is also necessary to evaluate the effect of continued submergence and different soil water patterns, on fertilizer interaction and chemical changes in rice soils, to understand the role of different soil water patterns on productivity. Experiments to get information on these aspects are needed. It may be mentioned that, while under irrigated conditions, fertilizer practice is comparatively easier, as various factors are at our control, in entirely rainfed areas, the conditions vary widely even on same soil type and one has to meet the prevalent conditions and recommend fertilizer practices to suit the occasion.

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CONDITIONS OF RICE GROWING SOILS AND MEANS OF IMPROVING FERTILITY BY APPLICATION OF MANURES AND FERTILIZERS

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Abstract

Researches on wet paddy nutrition were conducted in the Division of Agricultural Chemistry of the former Hyderabad Government during 1950–56, on sandy loam to loamy red earths and clay loam to clayey black soils.

Following are the findings:—

(I) Both the above two types of soils were found to be very deficient in nitrogen and phosphoric acid and paddy crop would not at all grow without phosphoric acid application.

(II) Although paddy crop (6 months crop) of an average weight of 4,000 lb. dry matter including paddy and straw takes up from one acre of the soil, only about 35 lb. of N, 9 lb. of P_2O_5 and 42 lb. of K_2O, the actual manurial doses of N and P_2O_5 are more.

(III) On sandy loam red earth, the optimum dose of N, was 60 lb. per acre and N: P_2O_5 ratio varied from 1 : $\frac{1}{2}$ to 1 : 1 with lower levels of N and at 60 lb. N level it was 1 : $\frac{1}{2}$. On B.C. Soil, the optimum dose of N was 45 lb. per acre and N: P_2O_5 ratio was 1 : $\frac{3}{4}$.

(IV) Growth studies of a long duration crop (6 months crop) showed that the greatest (nearly 67%) increase in dry matter and over 61% nitrogen and potassium absorption occurs during reproductive stage. In contrast, the absorption of P_2O_5 is very gradual and only about 28% takes place during reproductive stage and the maximum being during the tillering.

In the light of the above findings, field experiments conducted on times of application of manures indicated that in B.C. clayey soil N and P may be conveniently applied at puddle to obtain maximum crop returns for a short duration paddy (120–125 days). In case of light soil, there is need of split application of N, *i.e.*, half at puddle and half at 1st or 2nd weeding.

(V) Regarding method of application of fertilizers, smearing the seedlings roots with manure and mud-slush before transplanting gives

about 20% more paddy yields on heavy B.C. soil. But the same method is not suitable for light red earth soil.

(VI) *Other related factors affecting paddy nutrition.*—(*a*) Nitrogen and phosphoric acid applied through fertilizers (ammonium sulphate and superphosphate) to paddy crop are not appreciably lost from the root zone through vertical drainage.

(*b*) Paddy can tolerate a concentration of 0·2% soluble salt (NaCl) in irrigation water. Salt concentration between 0·2 and 0·5% affects paddy yields considerably, and above 0·5% is almost fatal to rice.

(*c*) Although paddy is grown in standing water, it needs subsoil drainage. Under ill-drained conditions, rice crop manured with fertilizers containing sulphate suffers from sulphide injury to the roots which get coated with iron sulphide due to reduction of sulphate and formation of sulphide.

(*d*) Paddy crop suffers if green manured under ill-drained conditions.

INTRODUCTION

DURING the period 1950–56, intensive studies on the nutrient requirements and other aspects of wet paddy cultivation in clayey black cotton soils and sandy loam to loamy red earths derived from acid rocks (granite) were conducted in the Division of Agricultural Chemistry, Department of Agriculture of former Hyderabad State. It is proposed to discuss here the most salient findings.

I. SOILS

As already mentioned, both heavy black cotton soils and sandy loam to loamy red earths were studied for wet paddy cultivation.

The black soils are generally finer and clayey in texture containing 40–50% clay and 12–22% silt. Usually they are calcareous, containing anything from 0·5 to over 5% free carbonates in the surface layers and their pH values range from 8·0–8·5. Their nitrogen and phosphoric acid contents are very low, *i.e.*, 0·02–0·04% only. The potash content is satisfactory, *i.e.*, from 1–2·5%. The clay minerals in these soils are predominantly of 'montmorillonite-beidellite' type and thus the exchange capacity of soils is high, *i.e.*, about 1 m.e. per gm. of clay.

The red earths are sandy loam to loamy in texture containing 15 to over 20% clay and 6–8% silt. Usually, they are non-calcareous and their pH values also are round about 7·0. These soils also are low in nitrogen and phosphoric acid contents which are of the same order of 0·03–0·04%. The potash content is generally quite high, *i.e.*, 2·5 to over 4%. The clay

minerals in these soils are predominantly of Kaolinite type and consequently their exchange capacity is about 0·5 m.e. per gm. of clay.

II. Nutrient Requirements of Paddy Crop

The following are the analytical data obtained in growth studies of the crop conducted at Rajendranagar Farm, Hyderabad-Dn.

TABLE I

Nutrients absorbed by paddy (6 *months crop*) *from one acre of soil* (*On oven-dry basis*)

Good average yield of dry matter lb.	N lb.	P_2O_5 lb.	K_2O lb.
4,000 ..	35	9	42

From the above data it is evident that paddy needs of N, P and K are not very high and especially, the P_2O_5 requirement is decidedly very low. But all the same the manurial doses of N and P to be applied for good yields of paddy are much higher than needed, as will be seen from further discussions.

In the early days of the " Grow More Food " drive, the cultivators could obtain very high yields by the application of only nitrogenous fertilizers, especially, oilcakes, as the soils were very deficient in nitrogen. But, with high paddy yields obtained season after season, the uptake of phosphoric acid from the soil was also more and eventually a stage was reached when application of only nitrogen was of no avail and the cultivators started complaining that the soils had gone alkaline. After pot and field experimentation, acute deficiency of phosphoric acid was revealed, so much so, that without P_2O_5 the crop would not grow at all. The findings were demonstrated on cultivators' fields. Some of the field data are given in Table II.

TABLE II

Field observation trial on black clay soil (*pH* 8·0)

Sl. No.	Treatment lb. per acre	Paddy yield in lb. per acre
1	Control (No Manure) ..	520
2	50 N only ..	150
3	50 P_2O_5 only ..	1,500
4	50 N *plus* 50 P_2O_5 ..	2,320

The above results revealed severe deficiency of P_2O_5 and also application of only nitrogen upsets the balance of N and P in the soil.

TABLE III

Paddy yield data on black clay soils (on cultivators' fields)

Site	Paddy yield per acre			
	No manure control	N 45 lb. per acre	P_2O_5 30 lb. per acre	N 45 lb.+ P_2O_5 30 lb. per acre
Rudrur (Nizamsagar) i ..	19	159	1,400	2,102
Rudrur (Nizamsagar) ii	11	43	1,324	1,964
Varni (Nizamsagar) ..	..	1,222	1,986	4,071
Humnapur (Nizamsagar)	..	163	1,159	1,962
Madhavnagar (Nizamsagar)	..	323	645	1,088

III. OPTIMUM LEVEL OF N AND N: P_2O_5 RATIO

Having established the deficiency of the soils in both nitrogen and phosphoric acid for paddy crop, the important and practical problems of N doses and N: P_2O_5 ratio to be used were studied. Pot culture experiments on sandy loam red earth having 7·3 pH and field experiments on black clayey soil (pH 8·0) were conducted for three seasons each with short-duration paddy H.R. 19 (120–125 days). Nitrogen doses of 15, 30, 45 and 60 lb. per acre with N: P_2O_5 ratios of 1: $\frac{1}{4}$, 1: $\frac{1}{2}$, 1 : $\frac{3}{4}$ and 1: 1 in each dose of N were tried.

The pot culture experiment on sandy loam red earth indicated that paddy yields significantly increased with increasing doses of N, *i.e.*, from about 2,400 lb. per acre with 15 lb. N, to over 4,700 lb. per acre with 60 lb. N level. The optimum N: P_2O_5 ratio at lower levels of N was between 1: $\frac{1}{2}$ to 1: 1 but at 60 lb. N level, it was 1: $\frac{1}{2}$.

The field experiments on B.C. soil showed that the optimum dose of N for the paddy variety was 45 lb. per acre and the optimum ratio of N: P_2O_5 was 1: $\frac{3}{4}$.

IV. TIME OF APPLYING OF MANURES

In case of applying artificial fertilizers like ammonium sulphate and superphosphate to paddy crop, the times of applying the manures had to be studied on both sandy loam red earths and clayey B.C. soil.

It may be pointed out that the times of applying the fertilizers depend upon (1) the rainfall in the growing season of the crop, (2) the soil type, (3) short or long duration of the paddy variety and (4) kind of the manures. Laboratory studies have shown that phosphates do not move through the soil and they may be applied all at one time at planting of the crop, thoroughly incorporated into the root zone of the soil. It is only nitrogen which may be lost through vertical and lateral drainage.

As already mentioned, growth studies were conducted with a long duration paddy variety H.R. 35, right from the sowing of the seed to harvesting stage to find out the amounts of N P K, absorbed by the crop during its different stages of growth, *i.e.*, seedling, tillering, intermediate and reproductive stages. The results revealed that the greatest (nearly 67%) increase in dry matter, and over 61% of nitrogen and potassium absorption occurs during the reproductive stage. In contrast, the absorption of P_2O_5 shows no such rapid increase and only about 28% of the total P_2O_5 absorption is during the reproductive stage and the maximum absorption is during tillering.

The growth studies incidentally also revealed that nearly 60% of the nitrogen taken in by the crop is found in the grain and the rest in the straw. Most of the P_2O_5 is translocated to the grain. In contrast to N and P almost 90% of potassium and 80% of calcium absorbed by the crop from the soil remains in the straw.

In the light of the above findings of growth studies, field experiments conducted on both types of soils helped to draw the following conclusions:—

In the case of heavy clay soil, both N and P may be conveniently applied at puddle to obtain maximum crop returns for short duration paddy H.R. 19. In cases of light soils, there is need of split application of N, *i.e.*, half at puddle and half at 1st or 2nd weeding.

V. Methods of Application of Fertilizers

Different methods of applying fertilizers were tried on clayey B.C. soil. The methods tried were (1) Broadcast and ploughed in dry before flooding and puddling, (2) Applied at last puddle, (3) All manure made into slush with mud and water and smeared to roots of seedlings before transplanting, etc.

Amongst the methods of application of fertilizers, the results showed that smearing the seedling roots with manure slush gives over 20% more paddy yields compared to all other methods using the same doses of N and P.

The method of smearing the roots was attempted as observation test in the case of sandy loam red earth soil, but it resulted in death of seedlings

after transplanting and thus the yields were depressed due to reduced population. Probably, this was because, the seedling roots were affected by the concentration of the manures which are not adsorbed by the soil due to low content and kaolinite type of clay. Thus this method of smearing the seedling roots is suitable for heavy soils only.

VI. OTHER RELATED FACTORS AFFECTING PADDY NUTRITION

(*a*) *Nutrient mobility in soils under swamp paddy cultivation.*—Laboratory test was conducted to study the movement of N added through ammonium sulphate to a porous sandy loam soil. The fertilizer was well mixed in the surface 2″ layer of the soil and the vertical movement of both ammoniacal and nitrate nitrogen through 10″ depth of the soil was studied after watering the soil for about 20 days till the water percolated through represented 22″ of rain-water. The results indicated that nearly 80% of the added nitrogen remained in the root zone, *i.e.*, within 6″ depth of the soil and the loss of ammoniacal N in 22″ of percolated water was only about 5·5% of the added nitrogen. Secondly, there was no loss at all of nitrate N in the leachate.

Similar laboratory experiment was conducted on movement of P_2O_5 added through superphosphate and leaching the soil with water representing 32″ of rain-water. The results showed that the movement of P_2O_5 was still slower and about 63% of the added P_2O_5 remained in the upper 2″ puddled layer and the total loss of P_2O_5 in the leachate was only below 2%.

(*b*) *Effect of saline waters on growth and yield of rice.*—Pot experiment was conducted to study the effect of salt concentration in irrigation water on the growth and yield of rice with sodium chloride salt varying from 0·05–1·0% concentration in the irrigation water which originally contained 0·03% total salts. The results indicated the following:—

(1) Rice can tolerate a concentration of 0·2% soluble salt (NaCl) in irrigation water.

(2) There is progressive decline in tiller and panicle counts and the paddy yield with increased concentration up to 0·5% salt.

(3) A concentration of 1·0% is fatal to rice.

(4) The above limits are in respect of crop grown in rainy season. Concentrations over 0·3% salt seem to be highly injurious to rice grown in summer season.

(*c*) *Drainage for rice fields* (*sulphide toxicity*).—Rice grown under heavy manuring with fertilizers containing sulphates, such as ammonium sulphate

and superphosphate was found to suffer in poorly drained soils, due to toxic effects of reduced products, *i.e.*, sulphide formed from sulphate. In order to verify this, pot culture experiments were conducted by using ammonium sulphate and urea for N, orthophosphoric acid for P_2O_5 and potassium chloride for K_2O. The N doses used were 0, 40, 80 and 120 lb. per acre with P_2O_5 and K_2O at half the nitrogen dose. The experiment was conducted both under drained and ill-drained conditions. The results have shown the following:—

(1) The roots of the paddy plant get coated with ferrous sulphide under ill-drained conditions and thus the crop dies due to lack of absorption of nutrients.

(2) The above trouble can be avoided by providing drainage of the soil or by using sulphate-free fertilizers.

(*d*) *Injurious effects of green manuring on rice under ill-drained conditions.*—The researches of the Japanese workers have shown the toxic effects of butyric acid and butyrates (which are among the products of decomposition of green manures) on paddy yields under ill-drained conditions. In order to test the views, pot and field experiments were conducted on sandy clay and black clayey soils respectively with application of varying doses (6,000–20,000 lb. per acre) of green matter with P_2O_5 at half the dose of N added through the green matter under drained and ill-drained conditions. The results confirmed the following conclusions:—

(1) Rice, though grown with standing water, needs subsoil drainage and ill-drainage reduce the yield of rice.

(2) Green manure applied to ill-drained and water-logged soils depresses the yield of rice appreciably, but opening of shallow drains helps to overcome this trouble.

References

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IMPROVING SOIL FERTILITY CONDITIONS WITH SPECIAL REFERENCE TO MINOR ELEMENTS

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Abstract

The micronutrient requirement of rice and the quantities supplied through various sources is discussed. Unlike in the other cereals, the quantities of trace elements supplied through water, rain and irrigation, is considerable.

The problem of Mn toxicity often met with in the new ayacuts (black soils) under Tungabhadra Project is discussed in detail and remedial measures are suggested. Particular attention is drawn to natural build up of organic matter and nitrogen in soil subject to rice cultivation. The role of organic matter in increasing the availability of iron is dealt with.

Visual symptoms, chemical analysis and tissue tests for diagnosing Mn toxicity are fully described.

The status of boron and copper in the black soils under Tungabhadra Project is still to be ascertained.

Introduction

Deficiency symptoms of micro-nutrients in rice have been described by Aiyer, Higginbotham and Patnaik under controlled solution culture experiments. Although response to application of trace elements has been obtained in several parts of India, notably, Kolaba and Ratnagiri areas of Bombay, Hebbal area of Mysore and at the Rice Institute in Orissa, the occurrence of typical and recognizable symptoms of deficiency seems rather rare under field conditions. Moreover, practically no data are available on either the absorption or the composition of the rice plant with respect to the trace elements.

Most of our information on trace elements therefore comes from the field response which, though of immense practical value, is of limited use for diagnostic purpose.

The present paper deals with some of the problems encountered in new ayacuts particularly in the black cotton soils under Tungabhadra Project. Except in passing, no attempt is made to cover the rest of rice growing areas of the Union, with which the author is admittedly unfamiliar.

Trace Element Requirement and Supply

Before discussing the subject proper, it would be of interest to gain an approximate idea of the trace element requirement of a rice crop and the amounts that would be supplied by the various manures and fertilizers commonly used.

The amount of nutrient taken up by a crop depends on the yield as well as the composition, both of which are likely to vary from tract to tract. The figures reported herein are based on the experience in the Tungabhadra Project area. Only the aerial parts are considered, as the stubble is normally left behind in the soil. An average yield of 2,000 lb. straw and 2,000 lb. grain is assumed.

	Fe	Mn	Cu	Zn	B	Mo ND
Composition in p.p.m.	200	200	10	10	3	..
Removed in lb./acre ..	0·80	0·80	0·04	0·04	0·012	..

Note.—Mo in rice is very low and with the facilities available at Bellary it has not been possible to determine it accurately.

Compared to other cultivated cereals, it is found that the demand of rice plant is heavy for Mn and rather small for B and Mo.

Sources and Quantities Supplied

(*a*) *Water.*—Unlike any other crop, rice needs much water either in the form of rain or irrigation. The contribution of trace elements from this source alone is substantial. In the calculation presented below, the composition of rain-water is assumed to be the same as reported by Hewitt except for Zn.

	Fe	Mn	Cu	Zn	B	Mo
Composition of rain-water p.p.m.	0·08	0·016	0·027	0·05	·013	·00075
Lb./acre from 30″ of rain	0·54	0·11	0·18	0·34	·090	·0051

The surface waters (lakes and reservoirs) and deep percolation waters (wells) are likely to contain less of Fe, Mn, Cu and Zn (by about 1/5) and

more of B and Mo (6–10 folds). Except for Fe and Mn, the entire demand for other trace elements could be met from this source alone.

(*b*) *Green manures.*—Wherever feasible, green manuring is a common practice. A normal dose is 5,000–6,000 lb. of green matter, that is, about 1,000 lb. of dry matter.

	Fe	Mn	Cu	Zn	B	Mo
Composition of green manure on oven-dry basis p.p.m.	160	250	13	24	200	0·3
Lb./acre per 1,000 lb. dry matter	0·16	0·25	0·013	0·024	0·200	0·0003

(*c*) *Oil-seed cakes.*—The average composition of groundnut cake and the trace elements supplied from 500 lb. of cake (equivalent to 30 lb. N) are given below:—

	Fe	Mn	Cu	Zn	B	Mo
Composition p.p.m. ..	580	25	17	63	40	10
Lb./acre from 500 lb. ..	0·29	0·0125	0·0085	0·0315	0·010	0·005

(*d*) *Cattle manure.*—The composition of ox-dung and urine is given below. For comparison, composition of human urine is also tabulated.

	Fe	Mn	Cu	Zn	B	Mo
Ox-dung dry basis ..	2,400	248	18	140	32	7·2
Ox-urine fresh basis ..	Tr.	Tr.	0·044	0·17	26·6	0·26
Human urine fresh basis	Tr.	Tr.	0·073	0·21	7·9	0·03

It is interesting to note that the animal absorbs a considerable portion of Mn and Cu, so that faeces is low in these elements compared to the feed ingested. Almost all the boron ingested is recovered in the urine. The differences between ox and human urine is to be found in the differences in the intake. Cattle take in large quantities of straw which is rich in B and Mo,

while the food of humans consists of grains which are rather low in these elements. For the same reason ox-urine is richer in K and N.

(*e*) *Wood-ashes.*—In the west coast on the laterite soils it is a common practice to apply wood-ashes. Wood-ashes supply not only the calcium and potassium which are so valuable but also contribute considerable amounts of trace elements as indicated in the following table:

	Fe	Mn	Cu	Zn	B	Mo
Wood-ashes of mixed spp. from red soils area	1045	165	75	160	146	1·83
Wood-ashes of *Porsopis julifora* from Hagari (black cotton)	2760	291	107	248	167	42·5

The enormous difference in Mo contents of the two ashes is noteworthy.

From the foregoing it is clear that the demand of rice for B and Mo is small and considerable quantities of these are supplied through water and manures. A deficiency of these elements in field is therefore extremely uncommon. Rather, cases of toxicity of boron might possibly occur in new ayacut areas.

Of the remaining four elements, namely, Fe and Mn; Cu and Zn the first set comes almost exclusively from soil, their availability being controlled by oxidation-reduction conditions in the soil rather than the amounts present. As a rule, the red loamy soils (chalks) are poor in manganese. The availability of Cu and Zn is governed by exchange and is much influenced by organic matter.

Trace Elements Problems in Tungabhadra Project Black Soils

(*a*) *Mn and Fe.*—The black cotton soils of the Tungabhadra Project are formed under low rainfall and high temperature. They are poor in nitrogen and phosphorus and contain large quantity of $CaCO_3$ nodules even in the surface (Krishnamoorthy *et al.*). The waters of Tungabhadra are of finest quality: low in salt with a favourable Ca/Na ratio. Complete analysis is as under:

Total salt	p.p.m.										
	CO_3	HCO_3	SO_4	Cl	Ca	Mg	Na	K	Cu	Zn	B
100	Nil	84	Tr.	19	16·6	4·7	22·4	Tr.	0·002	0·004	0·007

Even after correcting the nitrogen and phosphorus deficiency in soil, rice in new ayacuts exhibits characteristic symptoms. They are:—

In the early stages the symptoms are similar to those of P deficiency. The older leaves are most affected. Later, the drying of leaf advances down towards the base and from leaf margins to the midrib, so that in severe cases only the midrib remains green, the entire lamina bleached of chlorophyll. The dried leaf is whitish to yellowish white and does not show bronzing or browning as in P deficiency. Dark-brown streaks and spots sometimes visible. The youngest leaves may not show any characteristic symptom except perhaps drying at the tip.

That the symptoms are associated with Mn toxicity was proved by soil and plant analysis and reproduction of symptoms under controlled solution culture experiments.

Soil analysis.—The soils contain black nodules which, though are essentially carbonates of Ca and Mg, contain about 0·7% Mn (Desai). Under rainfed or light irrigated conditions, the exchangeable manganese remains at a low level of 1–2 p.p.m.

When such soils are grown to swamp rice, anærobic conditions set in. The manganese present in the higher states of oxidation is rapidly reduced to manganous state resulting in an enormous increase in the exchangeable Mn, as indicated in the following table.

	Ex. Mn p.p.m.	Easily reducible Mn p.p.m.
(i) Black cotton soil under rainfed cultivation ..	1·5	300
(ii) Normal paddy soil incubated anærobically for 30 days	47	550
(iii) Soil from affected incubation for 30 days ..	120	485

Leaf analysis.—The actual manganese content of the leaf is found to vary with age of the plant, position (physiological age) of the leaf. For example the manganese content of top leaves varies from about 350 p.p.m. in young leaves to about 100 p.p.m. in older plants. The absolute Mn content is, therefore, useless as a guide. Numerous leaf analyses conducted, however, indicate that the ratio of Fe to Mn is a better index. This ratio is close to 1·00 in normal leaves, the range being 0·8–1·20. In affected leaves the ratio may be as low as 0·16.

	Soil type	p.p.m. Fe	p.p.m. Mn	Fe/Mn
Set 1: Kunta area of Rajendranagar Farm				
Mature affected leaves	Black cotton	137	520	0·26
Mature normal leaves	Black cotton	90	137	0·72
Set 2: Dhadesgur Farm (Seedling stage)				
Affected leaves	Black cotton	135	938	0·16
Normal leaves	Black cotton	416	350	1·16
Set 3: Siruguppa area				
Completely dried lower leaves	Black cotton	110	2,795	0·04

Tissue tests.—Since the leaf analysis described above is time-consuming and requires elaborate laboratory facilities, a quick test for Mn toxicity was developed. Of all the tests tried, that described by Wallace is most satisfactory:—

0·5 gm. of fresh leaf tissue is shaken for 15 minutes with 5 ml. of Morgan reagent (NaAc + HAc PH = 4·8). To the extract 0·5 ml. of formal-doxime reagent is added and mixed followed by 2 ml. of 10% NaOH. The colour developed varies from deep wine red (Mn toxicity) to a faint brown to almost colourless (Normal).

Absorption of Mn and Fe by Rice

Because of general interest in Fe and Mn in the Tungabhadra Project, absorption of Fe and Mn by rice plant was studied (Desai *et al.*). Their results reveal that the entire requirement of manganese is absorbed during the vegetative phase of the plant. No significant amounts of Mn are absorbed during the reproductive phase. In this respect absorption of Mn is very similar to absorption of P and Ca.

The absorption of Fe is strikingly different. A large uptake is noticed during the flowering phase and absorption is sigmoid, closely following the yield curve and similar to absorption of N and K.

Interesting observations have been made on the fate of absorbed Mn when the manganese absorbed is in excess of requirement (Mn toxicity), there is the characteristic chlorotic drying of the lower leaves, in which the unwanted Mn accumulates. Such leaves are found to contain as high as

2,500 p.p.m. of Mn. These lower leaves which are no longer functional are shed away, thereby relieving the plant of unwanted manganese.

The influence of excess Mn on the absorption of other elements has also been investigated. There is an indication that in presence of large excess of Mn absorption of both P and N is retarded.

Effect of green manuring on the availability of Fe and Mn studied recently (Gopal Rao, 1956) offers a possible solution to the problem of Mn toxicity. Working with red loams (chalks) he noted that these soils hitherto under dry cultivation do not release enough iron when incubated alone and that the release of iron is greatly favoured by application of green manures. Within a short period of 30 days the available iron increased from 1 p.p.m. in control soil to about 1,000 p.p.m. in green manured. The beneficial effect of green manure is not marked in soils already under rice cultivation. Whether the same type of results would be obtained in black cotton soil rich in Mn is under study at Bellary.

When a soil is brought under rice the moisture regime is profoundly altered. The changes in the oxidation-reduction processes ensuing therefrom are well known. But perhaps what is not commonly appreciated is the fact that there is a considerable increase in organic matter and nitrogen content resulting directly from the change in moisture regime.

	N %	Organic carbon % (uncorrected)
Dry land 0–1′	0·021	0·30
1–2′	0·021	0·32
2–3′	0·021	0·26
Wet land 0–1′	0·055	0·87
(15 yr.) 1–2′	0·038	0·43
2–3′	0·034	0·38

The above results are in agreement with the work of Jenny. The objection that organic matter accumulates because of continuous manuring with organics is trivial. Results of permanent manurial plots under rainfed conditions do not support the contention.

The availability of Fe and Mn under swamp rice is a biological process and hence dependent on the organic matter. Moreover, as is well known manganese is much more easily reducible than iron and a sort of equilibrium exists between Fe and Mn.

$$Fe^{+++} + Mn^{++} \rightleftarrows Mn^{+++} + Fe^{++}$$

Further, in well drained soils the iron exists either in lattice of clay minerals or adsorbed on the clay particles mosaically. In both the forms iron is not easily available. Incidentally the mosaically adsorbed iron does not strictly behave as a cation and imparts property of anion exchange to the clay surface.

The low availability of phosphates under these conditions could easily be understood.

To reduce the iron and thus make it available, decomposable organic matter is necessary. It is pertinent here to recall that under swamp rice organic matter naturally builds up and the availability of iron improves in a couple of seasons. One may greatly hasten the process by green manuring. No one would have failed to observe the yellowish brown mottlings of iron in established rice soils. And with the reduction of iron, the availability of phosphorus also improves.

A point of interest that arises in this connection is the appropriateness of the term 'Mn toxicity'. Working with nutrient solutions Shive showed Mn toxicity is equivalent to iron deficiency. On analogy, it may be argued that what has been described in the foregoing is really a case of iron deficiency rather than Mn toxicity. Admitting the interdependence of Fe and Mn the term Mn toxicity is preferred for the following reasons:—

(1) Under field conditions, an excess of Mn does not always result in a deficiency of iron.

(2) The symptoms of Mn toxicity and iron deficiency are distinct both in character and site of affection.

(3) Analysis of affected tissues shows an abnormally high Mn content rather than an abnormally low iron.

Remedial Measures

The remedial measures consist of:—

(*a*) Addition of green manure to render the iron available,

(*b*) Opening up shallow drain to remove the Mn from the root zone of the plant,

(*c*) Absorption of P and N is restricted in the presence of excess of Mn. Liberal application of superphosphate to supply at least 50 lb. P_2O_5 per acre is essential. A top dressing with nitrogen at the pre-flowering phase appears beneficial. The results of a potculture experiment with split dose and times of application of ammonium sulphate are given below:—

Tr. No.	Particulars	Total tillers per pot		Grain yield in lb./acre (average of seasons)
		At peak tillering stage	At harvest (1954–55)	
1	No manure control	18	14	3,585
2	All nitrogen before planting	26	21	4,481
3	All nitrogen one month after planting	28	21	4,689
4	All nitrogen two months after planting	19	28	5,372
5	½ N at planting + ½ N one month after	26	19	4,402
6	½ N at planting + ½ N two months after	23	25	4,947
7	½ N one month after + ½ N two months after planting	23	23	4,440
8	⅓ N each at planting, one month and two months after planting	26	23	4,757

OTHER MICRO-NUTRIENT PROBLEMS IN TUNGABHADRA PROJECT

Boron.—The black soils of the area are rich in water-soluble boron, which generally increases with depth. The boron contents of a black soil long under rich cultivation (more than 100 years) under the old Tungabhadra Canals and another black soil nearby which is still cultivated rainfed are given below:—

	Dry land black cotton	Established wet land black cotton
	p.p.m.	
0–1′	0·47	0·30
1–2′	1·12	0·32
2–3′	2·06	0·34
3–4′	2·84	0·40

In a few cases as high as 1·20 p.p.m. B in surface has been recorded.

The high content of boron in dry soil is reflected in the boron content of rice grown on such soils.

Year of rice cropping	Boron content in 3rd mature leaf in p.p.m.
1st year ..	6·73
2nd year ..	4·80
15–20 years ..	2·94
100 years ..	2·90

The problem that awaits solution is whether:

(*a*) the "dry" soil contains boron enough to cause toxicity when the lands are brought under rice, or

(*b*) on continuous rice culture, the soil is depleted of boron and therefore boron fertilization would prove useful.

The symptoms of boron toxicity described by Mandal *et al.* (1956), namely, "little or no marginal scorching but a few dark and necrotic spots all over lamina", have not been observed even in the first season ayacuts. On the other hand practically no data are available on the response to boron application in the old ayacuts.

Copper.—The corrective effect of copper on excess of Mn is described in literature (Chapman). Further, as the organic matter content of soil increases, the availability of copper is depressed. The typical symptoms of copper deficiency, namely, wilting of the plant with marginal chlorosis of the young leaves (Aiyer and Patnaik) have not been observed in field. However a few of plant samples collected from Siruguppa Farm and other localities showed very low copper contents:—

	Years of cropping		Cu p.p.m.	Zn p.p.m.
1.	1st season	..	4·0	8·5
2.	2nd season	..	2·7	6·7
3.	15–20 seasons	..	1·8	8·5
4.	100 years	..	9·3	9·3

The first three samples are chlorotic and exhibit marked manganese toxicity symptoms. The fourth sample is drawn from a field which is known to yield 4,000 lb. of grain per acre with no visible symptoms of deficiency or toxicity.

No data on response to copper in black cotton of Tungabhadra Project are available.

The soils seem to contain enough zinc.

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THE PRESENT POSITION AND CONTROL OF RICE DISEASES IN INDIA

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Abstract

The diseases of rice are estimated to cause annually about 10 per cent. loss in rice production. The more important diseases are "blast," "helminthosporiose," "stem rot" and "foot rot". Of these "foot rot" can be checked successfully by treating the seeds with organo-mercurial fungicides and this method is now widely adopted whenever foot rot is prevalent in the country. "Blast" is the most destructive of the diseases and is of widespread occurrence. Research on the control of the diseases is directed towards evolution of highly resistant varieties, development of economic spraying and dusting schedules and the study of the factors favourable for disease development. There is need to intensify the programme of breeding resistant varieties in the country, as the cultivation of resistant varieties is the most economical method of control of the diseases. But since many resistant varieties are not presently available, plant protection by fungicidal spraying and dusting has to be resorted to, to save the crop against the damage caused by diseases. Economic spraying and dusting schedules have already been worked out for blast disease and they have to be popularised. For successful and large-scale adoption of plant protection measures, it is necessary to link the same with an efficient forecasting service. This has still to be organised in India.

Introduction

Of the various diseases reported on rice in India, the more destructive are "blast" caused by *Piricularia oryzæ* Cav., "helminthosporiose" or "brown eye-spot" caused by *Cochliobolus miyabeanus* (Ito *et* Kurib) Drechsler ex. Dastur (= *Helminthosporium oryzæ* B. de Haan), and "stem-rot" caused by *Leptosphæria salvinii* Catt. (*Helminthosporium sigmoideum* Cav. = *Sclerotium oryzæ* Catt.) and "foot-rot" caused by the fungus which has recently emerged into lime-light, *Gibberella fujikuroi* Saw. Recent reports go to show that the "udbatta" disease caused by *Ephelis oryzæ*, Syd. (*Balansia oryzæ* Nar. and Thirum.) is becoming destructive in parts of Bombay and in Jeypore tract of Orissa. Throughout Bengal, Bihar and in parts of Uttar

Pradesh and Madhya Pradesh a physiological root-rot complex is noticed in some seasons which tends to reduce the yield of crop.

Experienced plant pathologists like Thomas and Krishnaswamy (1948) have estimated that the diseases of rice might account for about 10% loss in rice production annually, or approximately 2·5 million tons. Epidemics of blast occurring in localised regions might cause up to 60–70% loss or even 100% in the case of individual fields. Even if the average loss caused by rice disease in a year were to be only 5% of the total rice production in the country, it would amount to a great deal, and prevention of such a loss should constitute one of the important methods of augmenting our meagre food resources.

The loss caused by the major diseases of rice, blast, helminthosporiose, and stem-rot, their mode of prevalence and control are discussed below:—

I. Blast Disease of Rice

(*a*) *Nature and extent of loss.*—Blast causes seedling blight in seedbeds. Severe leaf infection of blast occurring in the post-transplanting stage leads to total destruction of the foliage. At the neck-infection phase, blast is seen as a blackening extending a few centimeters on either side of the principal node in the rachis just below the ears. As a result of the infection, half-filled or totally chaffy earheads are formed and the earhead also tends to break, and fall off.

Serious losses in yield due to epiphytotics of blast disease have been recorded in different regions in India, such as Tanjore delta, Nellore, Hyderabad, Bombay, parts of Orissa, Kashmir and Kerala. In the first recorded outbreak of blast in India in 1918, in Tanjore, MacRae estimated the loss as 69% (Thomas and Krishnaswamy, 1948). In 1952, a seventy-five acre crop was completely wiped out in Deras Farm in Orissa. In 1955–56 season the early rices were totally destroyed in Kashmir, and about 70% of the remaining crop was severely damaged by blast. Under experimental conditions favourable for development of the disease, a loss of 43% in grain yield has been recorded in the Central Rice Research Institute Farm in a susceptible variety (Padmanabhan *et al.*, 1956).

Only large-scale plant disease surveys can give an objective estimate of the loss in yield caused by the disease in the country. The work of estimating crop losses is greatly facilitated if data are available to show the loss in yield the crop is likely to suffer under a given severity of infection. Studies made in this connection on blast disease have shown that the relation between loss

in yield and the neck-infection phase of blast outbreak could be expressed by the equation

$$Y = 1967 \cdot 95 - 18 \cdot 72\,X,$$

where Y = expected yield in lb. per acre and X = the estimate of the percentage of neck infection.

Based on this, it was estimated that for every one per cent. increase in neck infection in a susceptible variety there was a loss of 0·95% in yield. This relationship was worked out with a highly susceptible variety and it was anticipated that the relationship might be different with a less susceptible variety (Padmanabhan and Ganguly, 1955; Padmanabhan, Ganguly and Jha, 1958). Recently Rangaswamy and Subramanyan (1957) have confirmed that the loss in grain yield due to neck infection in susceptible and moderately resistant varieties was much higher than the loss in a resistant variety. Further studies are in progress to understand the relation between leaf infection and loss in yield with and without neck infection.

(*b*) *Epidemiology*.—The disease is reported to be seed-borne elsewhere but in India, though the spores of the pathogen have been seen to occur in the blackened kernels of the infected ears, no direct evidence is so far available to show that the disease is seed-borne. But alternative sources of infection might occur in nature. For instance, cross-inoculation studies carried out in Madras have shown that the isolates of the fungus occurring on *Panicum repens* and *Digitaria marginata* can infect rice (Ramakrishnan, 1948; Thomas and Krishnaswamy, 1948). Recently Pawar and Kulkarni (1954) observed that *Setaria intermedia* had leaf-spots resembling those of blast on rice and the causal organism was morphologically similar to *P. oryzæ*. *Dinebra retroflexa* has also been recorded as another collateral host in Andhra (Thirumal Rao, 1956). More disturbing was the recent report from Costa Rica (De Gutierrez, 1954) that the cultivated sugarcane was also a collateral host. How far these hosts help in the perennation of the fungus or serve as potential reservoirs of inoculum remains to be studied. The survival of the fungus in such grasses or in rice stubbles, perhaps in another phase of its life-cycle, is also a possibility.

Regarding the development of the disease, the susceptibility of the host and the weather are the more important factors as in the case of all diseases. With respect to blast, the host exhibits a fairly wide range of reaction under different conditions, perhaps much more so than in any other known disease. Apart from the variation in resistance shown by one variety as against another under identical conditions, which is varietal or genic in character the same

variety shows a range of reaction to blast in its development from seedling to maturity. The rice plant is most susceptible to leaf infection during the vigorous growth and tillering phase which follows transplanting (15–30 days after transplanting). As the plant grows older, it becomes progressively resistant to leaf infection (Padmanabhan and Ganguly, 1954); later at ear-emergence the plant becomes susceptible to nodal and neck infection. In the seed bed also the rice plant is in a vulnerable stage.

Besides the stages of its development, the level of N fertilization under which a variety is raised has a profound effect on its reaction to blast (Krishnaswamy, 1952; Padmanabhan, 1953; Ganguly, Balakrishnan and Padmanabhan, 1954). The rate of increase in susceptibility with increasing levels of N varies with different varieties, and this has been correlated with the accumulation of soluble N in leaf tissues under higher levels, the more susceptible the variety, the greater such accumulation (Hashioka, 1950; Baba, 1954).

The temperature at which rice is grown also alters the reaction of a variety to blast. Generally speaking, at temperatures below the optimum for the growth of rice, say at 20° C., the plant is more susceptible than when grown in the optimum range of 28–32° C. This has been related also to the N metabolism of the plant. At lower temperature the absorbed N tends to accumulate as soluble N in the leaf. Besides, it is reported that very little silica is absorbed under high level of N fertilization especially at lower ranges of temperature (Hashioka, *l.c.*).

Of the climatic factors, which favour blast development, the range of temperature (24–27° C.), a high relative humidity and copious dew formation appear to be the most important. Severe blast infection breaks out in the field when such favourable weather conditions occur when the host is also in a susceptible condition, or in a susceptible stage of development. There are thus two seasons in a year in Cuttack in which blast attains its maximum intensity, September–October in the main crop season and February in the second crop season. The varieties which flower during September–October get neck infection, while the late varieties still in the tillering stage during this period, get only leaf infection and escape neck infection. By delaying the planting, therefore, up to and beyond August, a severe blast incidence is induced, a fact which is utilized in the experiments on loss in yield, mentioned earlier. Similarly, in February, the crop gets generally severe leaf infection. In Madras, the weather is most favourable to blast development in December–January, when most varieties come to ear and thus become exposed to infection in the neck.

(*c*) *Control of blast disease of rice.*—Research on control of blast has been directed towards breeding of resistant varieties and development of economic spraying and dusting schedules for direct control. Data on the time of appearance of disease, the period of peak incidence and the effect of nitrogenous fertilization on various varieties can also be put to intelligent use in avoiding to some extent the loss caused by the disease.

The results so far obtained in India in breeding for resistance to blast have been recently summarised (Padmanabhan, 1956; Ghose *et al.*, 1956). Briefly, the results are as follows: At the Central Rice Research Institute, out of nearly 500 varieties, 14 have been selected as resistant to moderately resistant under artificial infection in the seedling stage and under all stages in the field under natural conditions. These have been tested in more than 40 Rice Research Stations all over India and except in rice stations situated at an altitude of 3,000′ or above they have been found to be more or less resistant in all centres. A second lot of 490 varieties from the genetic stock, all of which are early maturing—*i.e.*, within 110–20 days are being sifted for resistance at the Central Rice Research Institute and in the course of the next two years a few more resistant types will be made available from amongst the short duration types.

In Madras State, Co. 4 and T.K.M. 1 have been isolated as resistant types to blast. In Bombay, 15 improved varieties of the State have been reported to be resistant to blast. B.C.P. 1 and B.C.P. 2 are resistant selections from the Andhra State. H.R. 1 and H.R. 35 have been found to be comparatively resistant to blast in Hyderabad farms.

In hybridization projects to evolve resistant varieties, Madras is the only State which has released successfully resistant varieties, *viz.*, Co. 25, Co. 26, A.D.T. 25 and is in a position to release shortly a few more resistant selections like T. 6517 and T. 6522, T. 6538 and others. Recently G.E.B. 24 and Co. 4 have been crossed and a number of hybrids from the cross are showing great promise. A few selections from hybridization projects are in the final stages of testing in the Andhra State and in Bombay. From the Central Rice Research Institute, where, F. 6, F. 7 and F. 8 generations of plants are under study in crosses made between Co. 25 and Co. 13, a few high yielding early maturing, non-lodging and blast resistant types are likely to be made available within the next 2–3 years.

During the last two years, a very interesting International Co-operative Project is being carried out at Cuttack. This was the outcome of suggestions made in the meetings of the International Rice Commission by the Director

of the Central Rice Research Institute. Under this project rice varieties found resistant in different parts of the world are listed and distributed to several testing centres to study their reaction to blast under local conditions in the field and under artificial infection using standard procedures. A few susceptible varieties have also been obtained from each country and included in the tests. A standard procedure for testing and evaluating the reaction of the varieties on the basis of the extent of disease development has been suggested by the Central Rice Research Institute for this purpose.

The results obtained at the Central Rice Research Institute are given in Table I.

The classification of the varieties given in Table I is based upon the first year's trial and it is necessary to confirm the resistant reaction of the varieties by further tests. Further, the significance of the results to international breeding programmes will be apparent only when the results obtained in similar trials, conducted in other centres are available for comparison.

No real significance can, therefore, yet be attached to the differences between the reaction of the varieties in the home country and that noted in the Cuttack tests, though there are not many such differences. Out of the 14 susceptible types studied, 11 were found susceptible and 1 moderately susceptible. Similarly, in the case of the resistant varieties, only Bengawan and Sigadis were resistant amongst the 8 Indonesian varieties, but the Indonesian varieties were not definitely known to be resistant even in Indonesia. Out of the remaining 23 varieties, 22 proved to be resistant and moderately resistant. However, in the second crop season trial, only 10 out of 20 included were resistant.

In spite of the discrepancies noted above, the one important fact that clearly emerges out of the tests is that there appears to be a large reservoir of genic resistance to blast available in rice which can be successfully exploited to reduce the loss caused by blast in rice production in the world. Secondly, the general correspondence of the Cuttack results with the known reaction of the varieties in the countries of their origin suggests that the pathogen *P. oryzæ* does not occur as highly specialised pathogenic races as the rust and other fungi. Apart from specialisation in pathogenicity on the part of the fungus, the interaction of the host with the environment prior to its infection by the pathogen, is also an important factor determining the reaction spectrum of the host especially in a new environment.

Though control of blast through resistant varieties is the most economical method, it must be realised that the task of replacing the present lot of

TABLE I

Showing the list of resistant and susceptible varieties received from different countries which were tested at Cuttack under the International Co-operative Blast-Resistance Trials and their reaction to blast under Cuttack conditions

Reaction of the varieties included in the first crop season based upon artificial infection in seedling stage, and field reaction in post-transplanting (leaf blast) and in flowering stages (neck infection)			Reaction of rice varieties included in second crop season (January–April) based upon field reaction in post-transplanting stage (leaf blast only)		
Sl. No.	Name of variety	Reaction at Cuttack	Name of variety		Reaction at Cuttack
		(*A*) *Resistant Types*			
1	Nahada (Egypt)	MR			
2	Agami-Mont (Egypt)	R	Agami-Mont (Egypt)		R
3	Giza-14 (Egypt)	MS	Giza-a14 (Egypt)		MS
4	Co. 4 (India)	R	Shikare-Shiroke (Japan)		MR
5	Co. 26 (India)	R	Fujisaka 5 (Japan)		S
6	B.J. 1 (India)	R	Norin 17 (Japan)		R
7	S.M. 6 (India)	R	Kanto 51 (Japan)		S
8	S.M. 8 (India)	R	Sensho (Japan)		S
9	S. 67 (India)	R	P.I. 1 (Japan)		MS
10	A.K.P. 8 (India)	MR	Rei-Shi-Ko (Japan)		R
11	A.K.P. 9 (India)	MR	To-To (Japan)		S
12	Peta (Indonesia)	MS	Kwang-Fu-I (Taiwan)		R
13	Intan (Indonesia)	MS	Chia-Nong-Yo 242 (Taiwan)		S
14	Salak (Indonesia)	S	Chia-Nong-Yo 280 (Taiwan)		R
15	Tjahaja (Indonesia)	S	Norin 22 (Japan)		R
16	Sigadis (Indonesia)	R			
17	Remadja (Indonesia)	MS			
18	Tjina (Indonesia)	S			
19	Bengawan (Indonesia)	R			
20	Malik-Kuning (Malaya)	R			
21	Kontor (Malaya)	MR			
22	Seri Raja (Malaya)	R			
23	Mayang-Sagumpal (Malaya)	R			
24	Wag-Wag (Philippines)	R			
25	Raminad Strain 3 (Philippines)	R			
26	Zenith (U.S.A.)	R	Zenith (U.S.A.)		R
27	Lacross (U.S.A.)	R	Lacross (U.S.A.)		MS
28	C.I. 9045 (U.S.A.)	R	C.I. 9045 (U.S.A.)		R
29	C.I. 9075 (U.S.A.)	R	C.I. 9075 (U.S.A.)		MS
30	C.I. 9155 (U.S.A.)	MR	C.I. 9155 (U.S.A.)		R
31	C.I. 9249 (U.S.A.)	R	C.I. 9249 (U.S.A.)		MS

TABLE I—*Contd.*

Sl. No.	Reaction of the varieties included in the first crop season based upon artificial infection in seedling stage, and field reaction in post-transplanting (leaf blast) and in flowering stages (neck infection)			Reaction of rice varieties included in second crop season (January–April) based upon field reaction in post-transplanting stage (leaf blast only)		
	Name of variety		Reaction at Cuttack	Name of variety		Reaction at Cuttack
			(*B*) *Susceptible Types*			
1	Nabatat-Asmer (Egypt)	..	MR	Nabatat-Asmer (Egypt)	..	R
2	Sabaini (Egypt)	..	S	Co. 13 (India)	..	S
3	Co. 13 (India)	..	S	Norin (Japan)	..	S
4	A.D.T. 10 (India)	..	S	Kunshan-Wa-Shiang-Ken (Taiwan)		S
5	M.A.S. (Indonesia)	..	S			
6	Nachin 11 (Malaya)	..	S			
7	Siam 29 (Malaya)	..	MS			
8	Serendah-Kuning (Malaya)	..	MR			
9	Buenketan (Philippines)	..	S			
10	Thailand (Philippines)	..	S			
11	Caloro (U.S.A.)	..	R			
12	Fortuna (U.S.A.)	..	R			

Note.—R = Resistant; MR = Moderately Resistant; S = Susceptible; MS = Moderately Susceptible.

cultivated types by resistant ones is truly stupendous and will call forth herculean efforts sustained over a long period for achievement. The possibility of sudden mutation occurring in the pathogen which might set at nought all this effort in a trice is not to be overlooked. Therefore alternative methods have also to be evolved to supplement the results obtained by breeding. Studies made at the Central Rice Research Institute on control of blast by spraying fungicides have shown that the incidence of blast on even a very susceptible variety can be effectively reduced by spraying the crop with copper fungicides. The cost of chemicals involved is Rs. 40–50 per acre with the normal spraying rate of 100 gallons per acre, but only Rs. 10–12·50 with low volume spraying. (Padmanabhan *et al.*, 1956). There is, however, a distinct disadvantage in copper fungicides. Many rice varieties appear to be very sensitive to copper; they react adversely, showing severe symptoms of injury. In the absence of very severe disease the advantage of the protection given the copper fungicides cannot be appreciated.

The organo-mercurial dusts which are generally advocated as seed-dressings have been found to be very effective in checking blast infection

when sprayed or dusted, on the crop and they are not associated with any injury. The results of the last two years' results at the Central Rice Research Institute are so encouraging that it is anticipated that dusting with these chemicals may come to stay in India as a standard crop protection practice.

The success of any measure of control of crop diseases by direct application of fungicides depends largely upon timely action. That is to say, plant protection measures are really effective and popular only when they are linked with efficient forecasting service. Through forecasting the cultivators can be notified that conditions are sufficiently favourable for disease outbreaks, and that application of control measures will therefore result in economic gain, or, on the other hand, that the severity of the disease outbreak will not justify the investment involved in the control measure. Forecasting services have been successfully developed in the case of many plant diseases, notably, the late blights of potato and tomato, brown leaf rust (*Puccinia triticina*), vine mildew in France, Blackpod disease of Cocoa (*Phytophthora palmivora*) white-pine blister rust (*Cronartium ribicola*), apple and pear scab (Miller and O'Brien, 1952 and 1954; Anon, 1955).

The Japanese have successfully attempted forecasting blast outbreaks by studying the relationship between sunshine, precipitation and other factors during specific periods and the intensity of the subsequent blast outbreak (Kawada, 1954).

At Nagano Experimental Station, it has been found possible to forecast the degree of outbreak of neck rot and node blast by counting the number of spores " flying in the air " during the head distending stage.

Thirdly, it is also possible to forewarn the farmers about the imminence of blast outbreaks by watching carefully the pattern of disease development in highly susceptible varieties very early in the season. Thus by concerted work in all representative rice growing regions in India it is possible to develop a forecasting or forewarning service as the case may be, for blast, as well as other diseases. This will enable the taking of timely precautionary measures and in mobilising and concentrating all available resources in a region where they are most urgently required. It is to be hoped that it will receive sufficient attention from Agricultural Scientists in India in the immediate future.

The loss caused by blast in India can also be minimised by careful agronomic and cultural practices. Excessive N manuring, especially over 40 lb. N level, is to be avoided till resistant varieties which will not succumb to blast

at higher levels become available through breeding. Application of N in split doses should be encouraged. If incidence of blast in each region is carefully studied further it will be possible by some adjustment of planting dates and by choosing suitable varieties to avoid the coincidence of the most susceptible stage of the host with the peak incidence period in that region.

II. Helminthosporium Disease

(*a*) *Sources of infection and development of disease.*—The pathogen survives in the rice seed between harvest in November–February and the next sowing in June–July. However, it has been shown (Thomas, 1941; Padmanabhan *et al.*, 1953) that the seed-borne pathogen infects rice seedlings at germination only when the soil temperature is below 25° C. But the soil temperature in the main sowing season of rice in June-July is above 25° C., and therefore seed-borne infection is of minor significance during this period. The conidia of the fungus are air-borne and are present in the air throughout the off-season and may be responsible for the secondary infection in the seed-beds seen early in July (Padmanabhan *et al.*, 1953). Besides *Leersia hexandra* has been reported by Chattopadhyaya and Chakrabarthy (1953) to be a collateral host and whether this grass helps in the perennation of the fungus and serves as a source of infection for the newly sown crop remains to be investigated.

The range of conditions under which infection of the host takes place has been summarised elsewhere (Ling, 1951; Padmanabhan, 1956). It is of interest to note that contrary to what obtains in blast, the susceptibility of the rice plant to the disease increases with its age and it is most susceptible at flowering and maturing stages (Padmanabhan and Ganguly, 1954).

Serious epiphytotics of the disease have been indeed very rare, and in these years of outbreaks, unusual weather conditions have always been recorded. For instance, late and heavy rains in November and December and the floods which followed were said to have been responsible for first recorded outbreak of the disease in the country in the Godavari and Krishna delta (Sundararaman, 1922). In Indo-China rice was badly damaged by helminthosporium disease after late cold rains in December (Barat, 1931). In Ceylon, a severe outbreak occurred in 1943 and it was reported that unusually wet and humid weather had favoured the outbreak (Anon, 1943). Similarly, in the Bengal epiphytotic of 1942, heavy and unprecedented rains in September and, the floods they caused, were followed by continuous cloudy weather and by wet and humid November.

Helminthosporium disease appears to be very important in the highly humid heavy rainfall submontane areas below the Himalayas in Bengal, in

Eastern Uttar Pradesh, Assam and in the West Coast of India. It would be of great interest to examine how far the heavy rainfall and floods in these regions deplete the soil of its natural reserves of nutrients, especially when the crop is flowering and is most susceptible to infection. It may be mentioned that in the "AKIOCHI" phenomenon of Japan which develops under soils which get leached out of essential nutrients towards August–September, helminthosporium attack is very prominent. Paucity of K in soil, and in-balance of Fe/Mn ratio in the leaves are correlated with the severe appearance of the disease (Baba and Harada, 1954).

(*b*) *Control.*—The work in progress on the isolation of resistant varieties has also been recently summarised (Padmanabhan and Ganguly, 1953; Padmanabhan, 1956). In estimating the relative resistance of several hundreds of rice varieties, it was seen that it was futile to expect any high degree of resistance to the disease in any variety. There are five varieties, CH. 13, CH. 45, T. 141, Co. 20 and B.A.M. 10 isolated at the Central Rice Research Institute out of 500 varieties which do not show severe symptoms of disease under normal range of conditions. They may not behave as resistant under flooded conditions or under excessive heavy rainfall when the soil gets leached out of nutrients. A second dose of fertilizer application, after floods subside or when rains cease, may meet the case. As a matter of fact an experiment is in progress in Orissa in the Agricultural Farm at Bhubaneswar on a sandy soil which gets leached out during rains to test the above hypothesis.

III. Stem Rot

The sclerotia of the pathogen are hardy structures which survive in the old stubbles and straw and serve as the principal sources of infection of the new crop. They are carried from field to field through irrigation water and cause infection. In the case of this disease also excessive application of N predisposes the crop to infection. Therefore, one of the principal methods of avoiding loss caused by the disease is judicious application of fertilizers. Paracer and Luthra (1944) have also demonstrated that incidence of infection in the Punjab could be reduced by late sowing and late transplanting. Resistant varieties are known and their cultivation has to be taken up in areas where the disease occurs regularly to check the loss caused by the disease.

IV. Foot Rot

The loss caused by "foot-rot" can be kept under control by resorting to seed treatment with mercurial fungicides. "Foot-rot" was at one time

destructive, but wherever it is prevalent, it is kept under control by regular use of mercurial seed-dressings.

To conclude, prevention of the loss which amounts to 10% of our annual production, caused by rice diseases, is one of the more important and pressing problems needing immediate attention. Effective action is to be formulated and carried out both on the developmental and research side in order to conserve our principal food crop against the damage caused by diseases. The mode of prevalence and spread of the diseases are to be studied thoroughly so that the lacunæ which exist at present regarding these aspects are filled up. Based on such a knowledge scientific control through adoption of agronomical and cultural practices will become feasible. It is now well accepted that nitrogenous fertilization has to be resorted to, to increase the yield per acre of rice varieties, but as most of the released varieties succumb to " blast " or " stem-rot " under higher levels of N fertilization there is urgent need to breed new varieties, which will be resistant to these diseases under high fertility. A scheme for intensifying the breeding programme in this country is under consideration to evolve such blast-resistant types, and it is to be hoped that it will get started quite soon. Though cultivation of resistant varieties is ultimately the most economical method of control, alternative methods have also to be adopted till such varieties become available. Fungicidal spraying and dusting give adequate protection to the crop against " blast ", but the cultivators should be helped to make use of this knowledge. Forecasting and forewarning services are to be organized throughout the country if plant protection by chemicals is to become popular, because timely action before the pathogen establishes itself on the host is most effective and will also give adequate economic return by way of increased yield. Not the least important aspect of the problem is the necessity to manufacture such plant protection chemicals and equipment cheaply in the country.

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LATEST AND EFFECTIVE METHODS OF CONTROLLING INSECT PESTS ON RICE

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Abstract

With increased rice production under improved methods, the problem of plant protection is more accentuated. Insect infestation is found to be more in rice under high fertility. The usual indirect methods advocated till now, have not achieved the expected results. Chemical method of control appears to be the most effective at present. Insecticidal control of pests that feed on the foliage of the plant is comparatively easier. In the case of pests that remain concealed during their destructive phase, the choice of a suitable insecticide, the method and time of application of the insecticide seem to be the deciding factors in achieving effective control. At the Central Rice Research Institute, stem borers and gallfly have been effectively controlled by timing the application of insecticides to synchronise with the pest emergence. In the case of stem borers, a set of two sprayings is necessary at the time of brood emergence. The first spray kills the moths and checks oviposition while the second one, given 8–10 days later, kills the newly hatched larvæ before they bore into the stem. In the case of gallfly, spraying four times during the vegetative phase of the crop to synchronise with brood emergence reduced the incidence considerably. Yet insecticidal method can only be an adjunct or complementary to other methods of pest control. Biological control of these pests needs to be fully explored for a practical solution of the problem. Establishment of a pest warning organization is an immediate necessity to enable cultivators to take suitable pest control measures in time on a co-operative effort.

Insect pests cause such colossal damage to rice, both in the field and store, that protecting the crop and preserving the produce are no less important than stepping up rice production by improved seed or improved methods of cultivation. About 100 species of insects, with varying feeding habits, have been recorded as damaging rice in field and store. Not all pests are prevalent in one locality at the same time, as the abundance of a pest and the extent of damage caused, depend on the meteorological factors and cultural practices. Under favourable conditions, a minor pest in a certain

region may become a serious pest in the same region or in another region. Though, the actual loss caused by insects has not been assessed by statistical methods, it is estimated that insects cause 10% loss to the crop and stored grain. Saving this enormous loss depends upon devising and adopting effective and economic methods of control.

Methods of control consist of the adoption of simple prophylactic measures to avoid loss normally caused by pests and emergency measures aimed at fighting pest outbreaks. Hand-picking of egg masses, removal of dead hearts and destruction of hibernating pupæ in stubbles for stem borers; uprooting of infested plants for gallflies and mealybugs; systematic hand-netting for grasshoppers, leaf-hoppers and bugs; introducing insectivorous raptors for army-worms and cutworms are some of the methods of control which are not completely effective.

Breeding varieties resistant to insects is a complex problem involving an exhaustive study of insect-plant interaction factors and determining the exact factor responsible for resistance. Investigations at the Central Rice Research Institute indicate that pigment and scent in plants are associated with comparative resistance to gallfly. Breeding for evolving varieties bearing heritable characters for insect resistance is underway at the Central Rice Research Institute.

With the introduction of modern chlorinated hydrocarbon and organo-phosphorous insecticides, spectacular success has been achieved in the chemical warfare against pests. These insecticides are highly toxic at economic doses over a wide range of insects and possess greater residual toxicity. Yet, certain pests elude the efforts at control and limit the efficacy of insecticides by remaining concealed inside the central core of the stem. In order to control any pest effectively, a thorough knowledge of the biology of the pest is necessary to determine the vulnerable stage and time for control. Then, a toxic insecticide which at the same time is least hazardous has to be carefully selected. Some of the pests, like the swarming caterpillars, grasshoppers, stink bugs, leaf-hoppers, etc., which damage and feed on the exposed parts of the rice plant are comparatively easy for control. But, the internal feeders present a difficult problem for controlling even by the most toxic insecticide. Stem borers, gall midges, mealy bugs and root aphids are some of the pests which come under this category.

Some of the major pests of rice and their effective control are briefly described below;—

Stem borers.—The following five species of rice stem borers have been reported to occur in India causing anywhere from 3 per cent to 95 per cent loss in yield:

Schœnobius incertulas, Wlk.
Scirpophaga innotata, Wlk.
Sesamia inferens, Wlk.
Chilo simplex, Butler
Chilotræa (*Proceras*) *polychrysa*, Meyr.

The relative importance of the species of the stem borer varies with different regions but *Schœnobius* is more predominant throughout the country, particularly in areas under double cropping.

The characteristic symptoms of damage by stem borers are the presence of dead hearts at the early seedling stage and white earheads at the heading stage of the crop. It is usually presumed that the damage at the early seedling stage does not directly result so much in decreasing the yield, as the incidence at this stage has been observed to result in more tillers. It has been found that stem borer attack does result in profuse tillering, but many of the tillers thus formed do not contribute towards the yield as they remain green at the time of harvest. The loss due to stem borers at the early seedling stage has been estimated in an experiment to be 21·4 per cent.

Stem borer attack at the earhead stage of the crop is more apparent as many attacked earheads are rendered chaffy. The loss due to stem borers at the earhead stage of the crop has been estimated in the same experiment to be 26·9 per cent.

Insecticidal trials for the control of this pest have been in progress in the various States. BHC or DDT when sprayed or dusted was found to be effective in Bihar, Madras, Punjab and West Bengal.

In Andhra, Bihar, Mysore, Madras and West Bengal, the use of modern insecticides like Parathions and Endrin has contributed largely to the reduction in the borer incidence. However the dosage, time and the number of sprayings were different in each State.

The chief problem in the control of stem borers is that the egg masses of *Schœnobius incertulas* and *Scirpophaga innotata* remain covered by dense buff coloured hairs making insecticidal penetration difficult. The other factor in the biology of the stem borers is that the hatched out larvæ soon bore into the sheaths and stems and remain concealed while feeding. The larvæ of *Chilo* however nibble leaf-sheaths and flowers for sometime before

they would bore into the leaf-sheaths. On account of these habits of the pests, insecticidal treatment should be directed against the ovipositing moths and the newly hatched out larvæ. Experimental data collected at the Central Rice Research Institute on the efficacy of different insecticides, their dosage and time of application, indicate the following method to be the most effective in controlling stem borers:—Spraying in the nursery or transplanted or broadcast crop should synchronise with the prevalence of moths which can be determined by the catches at light-traps. This spray kills the adults and checks oviposition and should always be followed by another spray within a period of 8–10 days which is intended to kill the newly hatched larvæ. This schedule of spraying should be repeated according to the broods. At the Central Rice Research Institute, where there are two distinct broods, spraying Folidol 0·08 per cent according to the above schedule resulted in decreasing the stem borer incidence by 84 per cent and increasing the yield by 54 per cent over check. This schedule was also adopted by spraying 0·04 per cent Endrin in the cultivators' fields in a block of 100 acres with similar results. Spraying BHC or DDT at 0·2 per cent according to this schedule is also effective but as their residual toxicity is very low, the number of sprayings has to be increased.

It was seen that infestation by stem borers, leaf-rollers, leaf-hoppers, etc., was higher in the crop receiving high doses of nitrogen as ammonium sulphate. Therefore, there is a greater need to protect the crop receiving high doses of fertilisers.

Gallfly—Pachydiplosis oryzæ (W.M.) Mani.

This pest has been recorded from most of the rice-growing tracts in India and has been reported to cause a loss ranging from 15 per cent to even total destruction.

It is prevalent throughout the monsoon and at Cuttack the peak of infestation fluctuates between the end of August to the end of November. It has, on an average, 5–8 broods in the cropping season with considerable overlapping of generations. It has been found that the stage of the crop is an important factor in the incidence by gallfly as 90 days old crop showed higher infestation than the 60 days old crop.

Weeding out of alternate hosts and setting up light-traps to capture adults are commonly adopted in all the states. Growing early varieties which come to flower before the peak period of the pest has been recommended but such early varieties are generally poor in yield. Dusting BHC 10 per cent at the oviposition stage and spraying Folidol 0·04 per cent

or Endrin 0·02 per cent. in Andhra and Bihar respectively have been found to check the spread of this pest. The use of Parathion has proved promising in Bombay, Mysore and Madras.

The newly hatched grub does not wander much or feed outside but soon enters the primordium of the tiller and develops inside. The entire larval and pupal stages are spent in a single tiller. On account of these habits of this pest, insecticidal treatment by timing of the spray operations, as in the case of stem borers, is essential. It was found at the Central Rice Research Institute that spraying Folidol 0·08 per cent or Endrin 0·04 per cent four times during the vegetative phase of the crop, to synchronise with the brood emergence of the pest, reduced the incidence by 48·0 per cent resulting in an increase of 15 per cent in yield.

Dusting BHC or DDT is reported to control case-worms but often the cases containing the larvæ get dislodged from plants and remain afloat on water, as the larvæ are semi-aquatic. At the Central Rice Research Institute effective control has been obtained by spraying Folidol 0·08 per cent or Endrin 0·04 per cent and keeping the water impounded for 24 hours after the operations. Similarly, spraying Folidol 0·08 per cent or Endrin 0·04 per cent effectively controlled the caterpillars of leaf-rollers inside the webbed folds of leaf-blades. Grubs of hispa inside the leaf tissue and mealybugs inside the leaf-sheaths are effectively controlled by spraying Folidol 0·10 per cent.

Army-worms and cutworms are controlled by dusting 5 per cent BHC or DDT. A more effective method was found in spraying Folidol 0·08 per cent or Endrin 0·04 per cent as the larvæ died within four hours after spraying. The above method was also effective in controlling the other leaf-eating caterpillars.

Grasshoppers, leaf-hoppers and rice bugs could be effectively controlled by dusting 5 per cent BHC or DDT or Aldrin as both the adults and nymphs could be brought under check.

The success of the above methods of control depends on taking measures in proper time. It will be possible to forecast the onset of a pest from a knowledge of the environmental and climatic factors in relation to pest prevalence in different rice tracts and to issue a timely warning for control. This system of forewarning enables cultivators to take up control measures in proper time. Such pest warning is being adopted successfully in other countries, notably, Japan.

The above methods of control are only adjunct or complementary to other methods such as cultural, biological, etc. By extensive chemical control measures beneficial insects, parasites and predators of pests, are likely to be exterminated, thus presenting a fresh problem of increased pest incidence. In spite of 55 per cent egg parasitisation of *Schœnobius* and 89 per cent grub parasitisation of *Pachydiplosis* existing in nature, the ravages of the pests remain unabated. Biological control of these pests by breeding and releasing suitable parasites merits a serious all-out attempt. But until these methods are available, we have to say in the words of Wigglesworth "By all means let us use insecticides when we can do no better but we should regard them as admission of failure to be replaced by more subtle and more remunerative methods of biological control as soon as these can be worked out."

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SOME EVIDENCE AGAINST THE CHEMICAL THEORY OF NEUROMUSCULAR TRANSMISSION

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It is known that the different layers of the gastrointestinal tract contain acetylcholine (ACh), which is continuously released under physiological conditions and independently of the action of the central nervous system (Feldberg and Lin, 1949). The views about transmission at the neuromuscular junction are well known. It is generally believed that such transmission takes place by secretion of ACh or noradrenaline. This idea of neuromuscular transmission has not been generally accepted. According to Nachmansohn (1956), the mechanisms of conduction and transmission are essentially similar.

During estimations of ACh in the stomach muscle of the frog, *Rana tigrina*, it was noticed that though some muscles contained considerable quantities of ACh, none was detected in the eserinised saline in which the muscles were immersed and oxygenated at room temperature. No ACh was released from these muscles on nervous, chemical or electrical stimulation (Singh, Sharma and Bhatnagar, 1959). Other muscles continuously released ACh; in the present experiments, the release of this ACh was studied. Experiments were also performed on dog's stomach muscle.

Methods

These experiments were performed on the stomach muscle of the frog, *Rana tigrina*, and on dog's stomach muscle. Frogs were pithed and the stomach removed along with its nerves. It was cut along the greater curvature and its mucous membrane removed. It was then oxygenated in eserinised Ringer's solution for half an hour. It was next placed in a petridish in 12 c.c. of eserinised Ringer for one hour to note the release of ACh of the resting muscle. The Ringer's solution was then replaced afresh, and the nerves, which were placed on a pair of electrodes outside the saline, were maximally stimulated with induction shocks for 2 hours, but the Ringer's solution was replaced hourly for estimation of its ACh content. During the 4th hour, the muscle was placed untied in a small circular glass muscle chamber 1 cm. in diameter, having silver chloride electrodes at each end

for direct stimulation of the muscle and containing 12 c.c. of eserinised Ringer's solution. The muscle was thus stimulated for one hour with alternating current, 50 cycles per second, 10 volts for 10 seconds each minute. At the end of the stimulating period, the solution was tested for its ACh content. During stimulation the temperature of the saline rises by about 1–2° C., but the action of such rise of temperature was found to have a negligible effect on the release of ACh. These experiments were performed at room temperature, 23–25° C. The ACh was assayed on sensitive leech preparations. One litre of Ringer's solution contained 6·25 gm. NaCl, 0·15 gm. KCl, 0·265 gm. $CaCl_2$, 2 H_2O and 10 c.c. of 0·15 M $NaHCO_3$. Twelve gm. of eserine sulfate were added to 800 c.c. of the Ringer's solution.

Small dogs were chloroformed and the stomach along with its vagus nerves removed. It was cut along the greater curvature and its mucous membrane gently removed. A nerve-muscle preparation was made consisting of both the vagi and the cardiac half of the stomach. it was then suspended in a glass muscle chamber holding 60 c.c. of eserinised mammalian saline and oxygenated for half an hour. The mammalian saline was made in such a way that when diluted one and a half times, it gave the frog's Ringer solution for assay of ACh on the leech muscle, but it was not buffered as it contained excess of calcium. The muscle chamber had silver chloride electrodes at each end, as described by Winton (1926), Singh (1940) for direct electrical stimulation. When required, the muscle could be stimulated directly with alternating current, 50 cycles per second, 12 volts for 10 seconds every minute, or through its vagus nerves which were brought out of the chamber and placed on a pair of electrodes for maximal stimulation with induction shocks. After the preliminary oxygenation, the saline was replaced to note the secretion of ACh during resting state for one hour. It was then stimulated through the nerves for 1 or 2 hours, the saline being renewed hourly as with frog muscle. During the next hour, it was stimulated directly. These experiments were performed at 20–23° C., as at these low temperatures, the muscle liberates ACh at a constant rate for hours, and the variations in the rate of secretion thus easily recorded. The tension during contraction was recorded with an isometric lever.

The ACh content of the muscle was also determined in some experiments. Small dogs were chloroformed and the stomach rapidly removed; the mucosa was then gently dissected off. Pieces of muscle were cut out and washed with mammalian saline (Singh, 1940), and further aerated for half an hour at room temperature (20° C.) to remove any ACh sticking to them. When the washing was complete, pieces of muscle, used as controls, were soaked in mammalian saline for 2 hours. Other pieces were similarly soaked

in various experimental solutions. These experiments were performed at pH 7 (phosphate buffer), as at this pH the results were consistent. This may be considered as neutral point for activity, as tone decreases in acid and increases in alkaline solutions.

The extraction and assay of ACh was carried out by a method similar to that described by Richter and Crossland (1949). After experimentation, the muscle was transferred to a stoppered bottle kept in a freezing mixture, and was allowed to freeze and then weighed. It was then placed in 20 c.c. eserinised Ringer's solution which had been acidified with 0·1 N HCl to pH 4 and heated to 90° C. The muscle was cut into small pieces in this heated and acidified solution and kept at the high temperature for 5 minutes. The material was then cooled, centrifuged twice, and the supernatant fluid stored in a refrigerator. Before assay it was neutralised to pH 7·4 with 0·1 N NaOH. The extract was further diluted with eserinised Ringer's solution, so that 1 c.c. of the extract was equivalent to 50 or 100 mg. of the tissue.

The assay of ACh was carried out on an eserinised frog rectus muscle against solutions of known ACh concentrations, avoiding errors of sensitisation, as suggested by Feldberg (1948). Besides, the readings obtained by the experimental solution were well controlled by normal readings before and after its use. The difference in response of a rectus muscle to the repeated administration of the same sample or of the same standard solution was of less than 4%. These experiments were performed at 25° C., as at higher temperature, owing to leakage of ACh, the results were not consistent. Electrical stimulation of muscle was produced by stimulating with induction shocks. When the muscle was immersed in excess of potassium and ammonium ions, addition of calculated amounts of these ions which might have diffused into the muscle, to the control solutions before assaying on the frog rectus, did not make a significant difference to the results. All solutions were eserinised as above.

Results

Both frog's stomach muscle and dog's stomach muscle may continuously release ACh. The rate of release is very variable; some release copiously, some in traces and some none at all (Singh, Sharma and Bhatnagar, 1959). When the muscle is stimulated through its nerve, the mechanical response subsides in about 10–20 minutes owing to fatigue at the neuromuscular junction, but direct stimulation then causes a strong contraction. The secretion of ACh by the frog stomach muscle during resting condition, nervous stimulation and direct stimulation is shown in Table I. It will be

seen that during the first hour of nervous stimulation, there is invariably release of ACh which is diminished or absent in the second hour. But when the muscle is directly stimulated, there is enormous release of ACh, though nervous stimulation might have become ineffective. This shows that activity is a far more potent stimulus for release of ACh than nervous stimulation.

The next question arises as to the source of ACh during nervous stimulation. At first sight it would appear that the source is the neuromuscular

TABLE I

ACh released from frog's stomach muscle as a result of nervous and electrical stimulation

No.	ACh, nanograms, released from 1 gm. of tissue per hour						
	Resting	Nervous stimulation				Direct stimulation	
	1st hour	2nd hour		3rd hour		4th hour	
		ng.	Increase %	ng.	Increase %	ng.	Increase %
1	14	31	116	10	− 33	2,352	16,233
2	0	24	..	14	..	336	..
3	16	52	225	14	− 4	1,540	9,525
4	4	18	350	9	125	221	5,425
5	29	47	62	16	− 45	988	3,355
6	9	39	33	27	200	225	2,400
7	6	14	133	0	−100	500	8,233
8	0	6	..	4	..	340	..
9	3	7	150	3	0	267	10,150
10	0	4	..	0	0	400	..
11	38	50	32	42	11	168	342
12	14	44	214	31	121	280	1,900
13	29	99	241	58	100	234	707
14	50	142	184	86	72	271	442
15	30	39	30	32	7	177	490
16	41	81	98	64	56	151	270
17	18	21	17	8	− 56	42	133
18	11	18	64	7	− 36	120	1,082
19	19	19	0	15	− 21	209	1,000
20	40	66	65	44	10	154	285
21	11	36	227	14	27	666	5,956
22	3	4	33	2	− 33	300	9,900
23	18	30	67	22	22	81	350
24	20	19	−5	16	− 20	22	1,000
25	50	70	40	55	10	300	500

junction, and as it fatigues, the secretion of ACh becomes less, or is abolished. But it is also possible that the source of ACh during nervous stimulation is the muscle and not the nerves. This question was answered by experiments on dog's stomach muscle.

The results on dog's stomach muscle (Table II) are almost similar to those on frog's stomach muscle, but it has been found that if the muscle is stimulated through its nerves, there is release of ACh, but if the stimulation is stopped, then the release of ACh continues practically undiminished for several hours (experimental range up to 6 hours), and the tone increases. Thus in Table II, showing the effect of nervous stimulation during 2nd and 3rd hour, the ACh released during the 2nd hour of stimulation is practically the same as during the 1st hour. This would be whether the nerve is stimulated or not, so that the ACh could not have come from nerve endings; neuromuscular junction had long since fatigued during the 1st 10–20 minutes of stimulation as shown by the tension record. It appears that muscular contraction as a result of nervous stimulation released ACh; this then caused continued contraction with further release of ACh, a process of

TABLE II

ACh released from dog's stomach muscle as a result of nervous and electrical stimulation

No.	ACh, nanograms, released from 1 gm. of tissue per hour						
	Resting	Nervous stimulation				Direct stimulation	
	1st hour	2nd hour		3rd hour		4th hour	
		ng.	Increase %	ng.	Increase %	ng.	Increase %
1	32	69	116	..	..	117	453
2	27	23	−15	..	..	183	578
3	11	8	−23	..	..	86	782
4	34	95	183	..	..	907	2,619
5	79	118	49	..	..	281	253
6	0	0	0	..	..	Trace	..
7	40	59	48	..	..	471	1,078
8	56	160	186	160	186	520	830
9	42	85	102	85	102	233	450
10	33	85	158	85	158	1,579	4,655
11	46	104	126	104	126	1,104	2,300
12	8	14	75	11	38	110	1,275

continued self-excitation being set up. Thus it is clear that the most if not all the ACh liberated during stimulation of nerves comes from the muscle.

At 37° C., if the sodium of the saline is replaced with ammonium ions, then the muscle is drastically depleted of its ACh content (Table III). This might explain the mechanism of release of ACh. Ammonium ions are known to penetrate the muscle, and as ACh is a substituted ammonium compound, the ammonium ions probably displace it from combination. In the muscle, the mobilisation of potassium ions might be releasing ACh. At 25° C. potassium ions increase the ACh content of the muscle, but at 37° C., the action is the reverse, potassium ions decreasing the ACh content of muscle (Singh, Sharma and Bhatnagar, 1959 *a*). This is presumably due to increased leakage of ACh at higher temperature.

TABLE III

Effect of replacement of the sodium of the saline with ammonium ions on the ACh content of dog's stomach muscle; soaked for 2 hours at 37° C.

No.	ACh μg./gm. of muscle		
	Na	NH_4	Increase %
1	7·4	3·6	−51
2	6·8	2·6	−62
3	6·8	1·6	−76
4	7·4	3·0	−60
5	6·8	2·0	−70
6	6·6	1·3	−80

DISCUSSION

The most decisive evidence for the chemical theory of neuromuscular transmission has been the collection and identification of the transmitter after stimulation of nerves together with demonstration that the transmitter applied artificially can produce the characteristic muscular events and the potentiating effect of anticholinesterases. These experiments throw doubt on these findings. The chemical transmitter may come from the effector organ and

not the nerves. The present experiments leave no doubt that greater part of the ACh in neuromuscular excitation comes not from the nerve endings but from the muscle as a result of activity. It is doubtful if any ACh comes from the nerve, at least it may be no more than the small quantity involved in nervous activity and thus incidentally escape from the nerve endings. The nerve impulse releases the ACh from the muscle and the released ACh causes self-excitation of the muscle. The potentiating effect of anticholinesterases is thus explained. The so-called chemical neuromuscular transmission may therefore be nothing but autotransmission.

There is other evidence against chemical transmission at neuromuscular junction. The ions bromide, nitrate, iodide and thiocyanate have a differential effect on the response to electrical and chemical stimulation (Singh and Acharya, 1958). They ultimately decrease the response to electric current but increase that to ACh and potassium. The response to nervous stimulation decreases, thus resembling the response to electric current, but not that to ACh or potassium, suggesting that the response to nervous stimulation is due to electric current, that is, the action potential, or some unknown agency which acts like electric current but not like ACh or potassium. The response to nervous stimulation decreases, though these ions increase the release of ACh (Singh, Sharma and Bhatnagar, 1959 *a*).

Another salient feature in these experiments is the great variability in the amount of ACh released. Some muscles do not release any ACh under resting conditions, while others release comparatively copiously. Variable results are therefore to be expected, and this explains the discordant results obtained by other observers.

Summary and Conclusions

1. Some frog's stomach muscles and dog's stomach muscle continuously release acetylcholine.

2. Stimulation of vagus nerve increases the release of acetylcholine.

3. After the nerve has fatigued, direct electrical stimulation releases acetylcholine; this direct stimulus is far more potent than nervous stimulation, showing that the release of acetylcholine can occur independently of nervous stimulation.

4. In dog's stomach muscle, the release of acetylcholine continues undiminished after the nerves have fatigued. It occurs whether the nerves are further stimulated or not. This suggests that the acetylcholine released during nervous stimulation originates from the muscle. These experiments therefore throw doubt on the chemical tneory of neuromuscular transmission.

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BLOOD PROTEINS OF SOME INDIAN FRESHWATER FISHES

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An accurate analytical study of the protein make-up of the sera from different animals may reveal interesting species differences, apart from yielding valuable biochemical data. The earlier methods of protein separations by salting out and fractionation by organic solvents are both elaborate and tedious. The advent of electrophoretical methods as one of the most efficient tools of biochemical research has given a fresh impetus to the fascinating study of proteins and protein mixtures. Several workers (Deutsch and Goodloe, 1945; Moore, 1945; Geintz, 1954) have studied the protein patterns of a number of animal species using boundary and zone electrophoretic techniques. The serum protein fractionation of some animals of relatively lower phylogenetic order has been reported by Deutsch and McShaw (1949). Giri and Pillai (1957) have reported the serum protein composition of twelve common animal species—elephant, horse, buffalo, camel, cow, goat, sheep, dog, rabbit, rat, guineapig and man. Few workers have undertaken the study of blood serum proteins of fish in any detail. Andre Drilhon (1953, 1954 *a*, *b*; 1955, 1956) has, however, reported the qualitative composition of the serum proteins and lipoproteins of eel and carp. But no information on the quantitative or qualitative composition of blood sera of Indian freshwater fishes is available in literature. Hence, a detailed investigation on the protein make-up of the blood sera of five common species of Indian carp belonging to *Cyprinidæ* family has been undertaken in our laboratories and the data are presented in this communication.

Experimental

Blood samples were collected fresh from the heart after dissection of the live fish, into clean dry pyrex glass tubes and allowed to clot at 37° C. for one hour. The samples were then centrifuged at 2,500 r.p.m. for 15 minutes and the clear sera obtained were used for subsequent analysis.

Serum protein analysis.—The serum proteins were analysed by the agar electrophoresis method [Giri and Pillai (1956) and Giri (1956)]. A suitable

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volume (30 μl.) of the serum samples was applied by the paper strip application technique on the surface of the agar gel (0·5 per cent., B.D.H. fine powder) and the electrophoresis carried out in veronal acetate buffer (pH 8·6, μ 0·05) at 220 v., 5 m A. After 4 hours' run the electrophoresis was discontinued, the agar plate was dried and stained with Amido schwarz 10 B (E. Merck). The different protein components could be seen as blue bands on a colourless background and their percentage distribution was calculated from the electropherogram obtained by use of a photovolt electronic densitometer (Photovolt Corporation, New York, Model 52–C) and by measuring with a planimeter the area covered by the different protein components indicated by the separate peaks in the electropherogram (Giri, 1956).

Lipoproteins.—30 μl. serum was spotted and the electrophoresis carried out exactly as in the previous case. The dry agar plate was stained with Sudan Black according to the method of Swahn (1953). 0·5 gm. of Sudan Black was dissolved in 500 c.c. of 60 per cent. ethyl alcohol, heated just to boiling, cooled, filtered and stored. The agar plates were stained in the Sudan Black solution for 30 minutes and washed with 50 per cent. ethyl alcohol for 2 minutes, twice. The lipoproteins were visible as blue back bands on a colourless background and were estimated by use of a densitometer and plannimeter, as in the case of serum proteins.

Mucoproteins.—Analysis of fish sera for mucoproteins was carried out by the method of two-dimensional electrophoresis (Giri, 1957), and by the staining procedure of Barrollier and Watzke (1956).

Total proteins were estimated by micro-Kjehldahl method. The serum nitrogen values were multiplied by the factor 6·25 and was taken as the total protein content.

The reliability of the method of serum protein estimation by agar electrophoresis technique was checked up and confirmed by carrying out a number of duplicate experiments. Out of the 25 duplicate estimations carried out, 72 per cent. of the values showed a maximum difference of only 1 per cent. Even of the rest of the values which showed higher differences, the maximum difference observed was 5 per cent., and that too was noted only in one case. The standard error for duplicate measurements was found to be 1·3 per cent., which was considered negligible.

RESULTS

Five common species of freshwater fish, Catla (*Catla catla*), Rohu (*Labeo rohito*), Mrigal (*Cirrhina mrigala*), Bata (*Labeo bata*) and Kalbaus (*Labeo calbasu*) were investigated. A clear separation of five components

albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin was obtained in all the fish sera samples. Though the five different species did not exhibit any qualitative differences in their protein composition there were appreciable quantitative differences in the different protein components present. Table I shows the percentage composition of the five different serum protein components as well as of the total serum protein per cent. in the five species. In Fig. 1 is given the photograph of typical electrophoretic patterns of fish serum from the five different species as compared with that of normal human serum.

The fish sera on analysis for lipoproteins yielded a very clear separation of at least two components α-lipoprotein and β-lipoprotein in all the species. In the Mrigal, there was an additional component with higher electrophoretic mobility than that of α-lipoprotein and this component is designated here as α_F-lipoprotein. Figure 2 shows the photograph of the electrophoretic patterns of the serum lipoproteins in the five different species as compared to that of normal human serum. In Table II is given the composition of lipoprotein in fish sera expressed as percentage of total lipoproteins.

The analysis of mucoproteins of fish sera did not, however, reveal any observable differences between the species.

To evaluate the differences among the mean values of the 5 species on each characteristic, analysis of variance was done. Table III shows that in one characteristic, that is α_2-globulin, the 5 species do not differ significantly from one another suggesting that they can be taken as random samples from a common normal population. In the rest of the seven characteristics (Albumin, α_1-globulin, β-globulin, γ-globulin, total proteins, α-lipoprotein and β-lipoprotein) significant differences among the five species are observed; *i.e.*, in each of these cases the five species cannot be taken as random samples from a normal population.

Since the analysis of variance showed that on characteristics albumin, α_1-globulin, β-globulin, γ-globulin, total protein, α-lipoprotein and β-lipoprotein the 5 species differ significantly among themselves, t values (two-tailed) were calculated with a view to assess whether any two particular species differ significantly from one another in their mean values. The t values are shown in Tables IV and V.

Discussion

Though the general protein pattern looks similar in all the five species a perusal of Tables I and III would show that appreciable interspecies variations do exist. The albumin values of all the species are nearly similar,

TABLE I

Protein distribution in freshwater fish serum

(Expressed as percentage of total proteins)

Species	Albumin		α_1-Globulin		α_2-Globulin		β-Globulin		γ-Globulin		Total serum proteins gm. percentage	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
C. catla	42·58	9·27	8·83	3·51	15·17	4·36	23·00	2·71	10·42	5·77	3·11	0·45
L. rohito	50·17	10·60	17·42	8·02	13·21	7·19	7·17	3·64	12–04	8·19	2·86	0·46
C. mrigala	47·26	10·62	30·26	10·48	11·91	7·30	6·09	3·32	4·96	3·07	2·69	0·34
L. bata	58·60	6·51	16·67	5·06	12·53	3·91	5·33	2·39	6·47	3·79	2·51	0·44
L. calbasu	41·50	10·56	25·75	7·77	16·12	6·29	10·25	3·38	6·88	5·44	2·61	0·21

excepting that of Bata wherein it is the maximum (58·6 per cent.). On this component, the *t* values between Bata and Kalbasu, Bata and Mrigal, and

TABLE II

Lipoproteins in Indian freshwater fish sera

(Expressed as percentage of total lipoproteins)

Species	α_F -Lipoprotein		α-Lipoprotein		β-Lipoprotein	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C. catla			53·11	18·89	46·89	18·89
L. rohito			77·8	7·88	22·2	7·88
C. mrigala	7·73	3·49	39·06	7·67	53·20	7·43
L. bata			35·2	11·56	64·8	11·56
L. calbasu			71·80	8·91	28·20	8·91

TABLE III

Table showing analysis of variance of five species on the different blood protein values

Characteristic	Sum of squares		df		Mean square		F
	Between groups	Within groups	Between groups	Within groups	Between groups	Within groups	
Albumin ..	2428·40	7846·30	4	77	607·10	101·90	5·96†
α_1-Globulin ..	4493·44	5086·76	4	77	1123·36	66·06	17·01†
α_2-Globulin ..	158·92	3240·06	4	77	39·73	42·08	0·94
β-Globulin ..	2840·25	835·99	4	77	710·06	10·85	65·44†
γ-Globulin ..	713·54	2677·44	4	77	178·38	34·77	5·13†
Total proteins ..	2·33	8·54	4	47	0·58	0·18	3·22*
α-Lipoprotein ..	13958·67	6449·82	4	44	3489·50	146·59	23·80†
β-Lipoprotein ..	11551·40	6395·29	4	44	2887·85	145·35	19·87†

Note.—* Significant at 5% level. † Significant at 1% level.

Bata and Catla are significant at 1 per cent. level and that between Bata and Rohu at 5 per cent. level. The α_1-globulin value is the lowest in Catla and the highest in Mrigal. The *t* ratios between α_1-globulin values of Catla and the rest of the four species are significant at 1 per cent. level. The values between Mrigal and Bata, Bata and Kalbaus and Rohu and Mrigal are also significant at 1 per cent. level. The α_2-globulin does not exhibit any significant concentration variations amongst the different species. It is noteworthy that the analysis of variance (Table III) also shows that on α_2-globulin, the five species do not differ significantly. The very high concentration of β-globulin (23 per cent.) in Catla is noteworthy. The *t* values of this component between Catla and rest of the species are all significant at 1 per cent. level. Of the other species, only Kalbaus shows *t* values significant at the same level of 1 per cent. when compared with Bata and Mrigal. In evaluating the β-component, extreme care has to be taken to employ unhæmolysed serum for analysis, lest the fish hæmoglobin which has similar electrophoretic mobility to that of β-component vitiates the β-values. Catla and Rohu are different from the rest of the species in their higher γ-globulin concentrations. The *t* values of γ-globulin values between Catla and Mrigal and Rohu and Mrigal are significant at 1 per cent. level. On the total serum protein which is highest in Catla, the *t* value between Catla and Bata is significant at 1 per cent. level and that between Catla and Mrigal and Catla and Kalbaus, at 5 per cent. level.

In lipoprotein composition, the Mrigal varies from all other species with respect to the presence of one additional component (α_F), which has a higher electrophoretic mobility than that of α-lipoprotein. Though this component is present only in a small concentration (7·73 per cent.), this factor seems to be a distinct feature of the Mrigal species. In the rest of the species only two components (α-lipoprotein and β-lipoprotein) could be clearly observed. The α-lipoprotein concentration is maximum in Rohu and minimum in Bata (Table II). On both α- and β-lipoproteins the *t* values between Catla and Rohu, Rohu and Bata, Rohu and Mrigal, Mrigal and Kalbaus and Bata and Kalbaus are significant at 1 per cent. level (Table V). On β-lipoprotein the *t* value between Mrigal and Bata is significant at 1 per cent. level. On both α- and β-lipoproteins, *t* value between Catla and Bata is significant only at 5 per cent. level. The *t* values between Catla and Kalbaus and Rohu and Kalbaus are insignificant on both α- and β-lipoproteins.

A close study of the general feature of protein distribution in fish sera reveals that it shows considerable variations from the mammalian sera. It may be interesting to note in this connection, that there are about 18,000

TABLE IV

Table showing t *ratios between the serum proteins of different species of freshwater fish*

Sample size	Species	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin	Total proteins	
							t value	Sample size
12	*C. catla* vs.	0·23	6·24†	0·38	8·85†	1·31	2·59*	12
8	*L. calbasu*							6
12	*C. catla* vs.	5·05†	4·40†	1·59	17·32†	2·06*	3·05†	12
15	*L. bata*							10
12	*C. catla* vs.	2·05*	3·45†	0·84	12·98†	0·60	1·29	12
24	*L. rohito*							12
12	*C. catla* vs.	1·25	6·68†	1·38	14·83†	3·55†	2·53*	12
23	*C. mrigala*							12
24	*L. rohito* vs.	1·94	2·48*	0·93	2·04*	1·61	1·11	12
8	*L. calbasu*							6
24	*L. rohito* vs.	2·70*	0·32	0·33	1·69	2·41*	1·62	12
15	*L. bata*							10
24	*L. rohito* vs.	0·92	4·64†	0·60	1·04	3·81†	0·92	12
23	*C. mrigala*							12
23	*C. mrigala* vs.	1·28	1·07	1·40	2·95†	1·19	0·47	12
8	*L. calbasu*							6
23	*C. mrigala* vs.	3·61†	4·56†	0·29	0·74	1·31	0·97	12
15	*L. bata*							10
15	*L. bata* vs.	4·58†	3·23†	1·60	3·87†	0·20	0·46	10
8	*L. calbasu*							6

Note.—* Significant at 5 per cent. level (two-tailed). † Significant at 1 per cent. level (two-tailed).

TABLE V

Table showing t *ratios between the lipoproteins of different species of freshwater fish*

Sample size	Species	α_1-Lipoprotein	α_2-Lipoprotein
9–5	*C. catla* vs. *L. calbasu*	1·93	1·93
9–10	*C. catla* vs. *L. bata*	2·51*	2·51*
9–10	*C. catla* vs. *L. rohito*	3·58†	3·58†
9–15	*C. catla* vs. *C. mrigala*	2·44*	1·11
10–5	*L. rohito* vs. *L. calbasu*	1·24	1·24
10–10	*L. rohito* vs. *L. bata*	9·61†	9·61†
10–15	*L. rohito* vs. *C. mrigala*	11·70†	9·57†
15–5	*C. mrigala* vs. *L. calbasu*	7·50†	5·87†
15–10	*C. mrigala* vs. *L. bata*	0·96	2·93†
10–5	*L. bata* vs. *L. calbasu*	5·79†	5·79†

Note.—* Significant at 5 per cent. level (two-tailed). † Significant at 1 per cent. level (two-tailed).

known species of fish as compared to about 3,500 known species in mammals (Dobzhansky, 1951). The total serum proteins in fish are also significantly low as compared to that of the mammalian species. This finding is in agreement with the observations of Andre-Drilhon (1954 *b*; 1955) who had reported the serum protein patterns of eel and carp. In the five common species of Indian freshwater fishes studied, considerable variations are also found to occur between the species. Stansby (1954) has reported a similar finding of interspecies variations, in his study on the freshwater fishes in the United States. Considering some of the other characteristics as the size, rate of growth and feeding habits of these species the differences in their blood protein pattern is only to be expected. A similar study on the rest of the species, especially those of the marine fishes is bound to reveal interesting informations about the blood protein patterns of the fish species and their correlation to environmental and other factors.

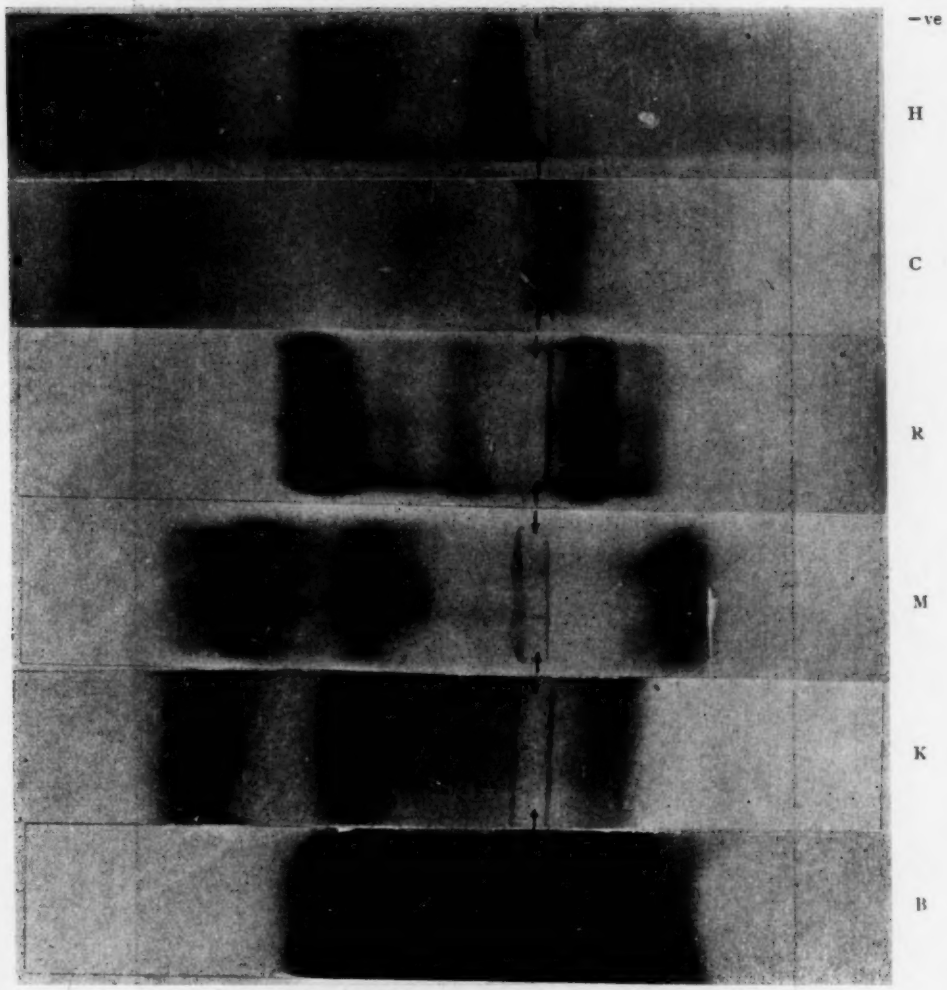

FIG. 1. Electrophoretic patterns of fish sera as compared to normal human serum. The arrow indicates the point of application of serum

H = Human serum; C = Catla; R = Rohu; M = Mrigal; K = Kalbaush; and B = Bata

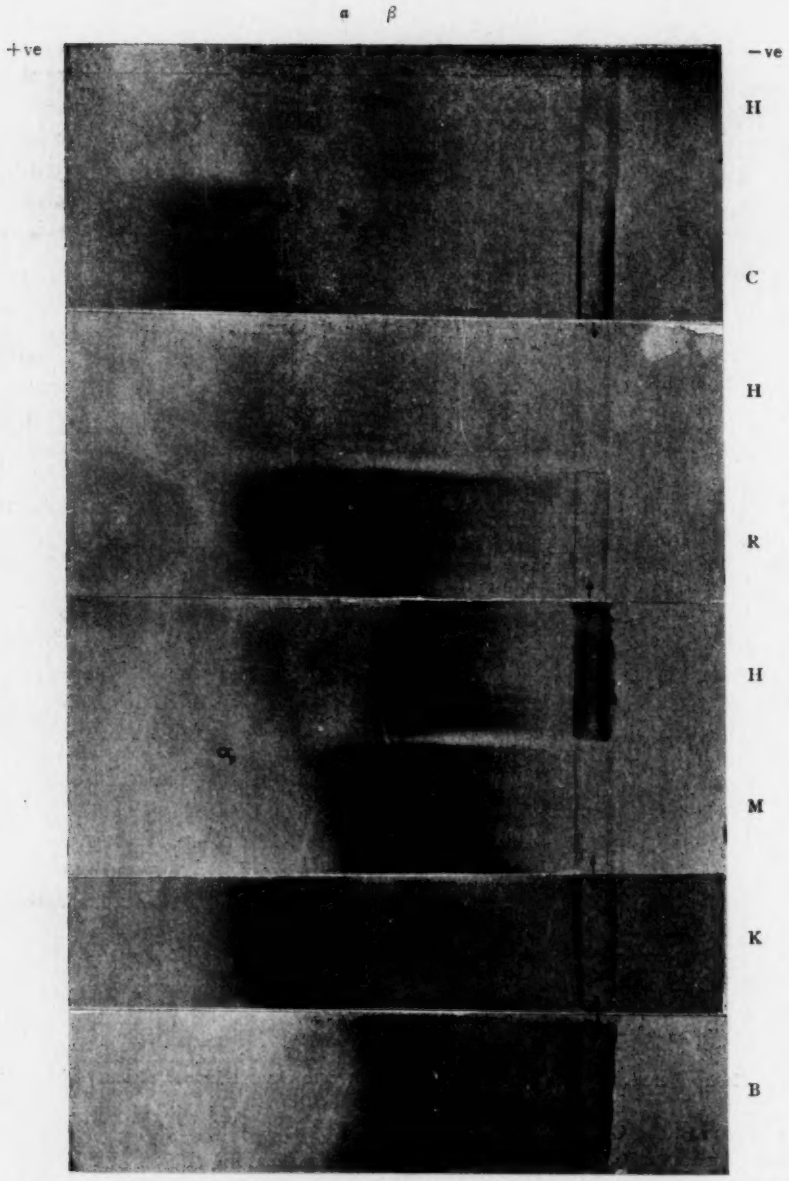

FIG. 2. Electrophoretic patterns of lipoproteins in fish sera and human serum. The arrow indicates the point of application of sample

H = Human serum; C = Catla; R = Rohu; M = Mrigal; K = Kalbaush; and B = Bata

Summary

A study of the blood serum proteins and lipoproteins of five species of India freshwater fish (*Catla catla, Labeo rohito, Cirrhina mrigala, Labeo bata* and *Labeo calbasu*) has been carried out, by the agar electrophoresis method. The blood protein pattern of fish is significantly different from that of other mammalian species. Though the general pattern of the five species of fish studied appear to be similar, appreciable interspecies variation is found to exist in the blood protein make-up of freshwater fishes.

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STUDIES ON THE NUTRITION OF RICE PLANT (*ORIZA SATIVA* L.)

Part V. Comparative Effect of Ammonium and Nitrate Nitrogen on Growth, Yield and Nitrogen Uptake by Rice Plant

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Relative merit of ammonium and nitrate nitrogen, as a source of nitrogen for growth of rice plant, grown under solution and sand culture technique, has been examined by a number of workers. Espino and Estioko (1931) and Dastur and Malkani (1933) considered a mixture of ammonium and nitrate nitrogen as better nitrogen source for rice, than either used alone. Thelin and Beaumont (1934) reported that for better growth of rice, the mixture should have a higher proportion of nitrate to ammonium nitrogen. Gericke (1930) and Asana (1945), on the other hand, reported satisfactory growth of rice with nitrate alone as a source of nitrogen. Asana (1945) also reported that it was not possible to grow healthy rice plants for long in ammonium cultures alone at low pH.

Under field conditions in the submerged soil state, however, ammonium nitrogen has been found to be a more efficient nitrogen source for growth of rice crop. Nagaoka (1904), Daikuhara (1905), Kelley (1911) and Harrison (1912), quoted by Espino (1922), reported that ammonium nitrogen was more effective for increasing rice yields than nitrate nitrogen. Dastur and Pirzada (1933), on the other hand, obtained better growth of rice with a mixture of two forms of nitrogen. Dastur (1940), however, reported that a mixture of ammonium sulphate and sodium nitrate was superior to ammonium sulphate in a few trials only but was either equal or inferior to ammonium sulphate in other trials. Recently, Abichandani and Patnaik (1958), summarising a large data from field experiments on the efficiency of different nitrogenous fertilizers concluded that ammonium fertilizer was far superior to nitrate fertilizer for increasing rice yields and that the fertility value of a mixture of the two forms was intermediate between the two and depended on the relative proportion of ammonium to nitrate nitrogen in the mixture.

In view of this difference of opinion, investigations were undertaken to re-examine the question of suitability of ammonium and nitrate nitrogen for growth and yield of rice crop. Studies were first conducted under solution culture technique, free from nitrifying and denitrifying influences met with in soil culture. Results of these investigations are summarised in the present communication.

Material and Methods

Rice variety *Ptb* 10 of 110 days duration was grown under solution culture technique, described earlier by Tanaka *et al.* (1958 *a*). Seeds were sown on June 16 and seedlings were transplanted in the solution culture pots on July 11. Two nitrogen levels, 20 and 100 p.p.m., were tried. Twenty p.p.m. was considered as moderate level for plant growth and 100 p.p.m. as high level (Tanaka *et al.*, 1958 *a*). Ammonium nitrogen was given in the form of ammonium sulphate and nitrate nitrogen was given as sodium nitrate. Three seedlings were transplanted per pot and culture solution in the pots was changed every seven days.

Initial pH of both ammonium and nitrate cultures was maintained at 4·8. Because of unbalanced uptake of ions from the culture solution (ammonium being cation and nitrate being anion), the pH of ammonium culture solution dropped by about 0·2–0·4 pH units and that of nitrate culture solution increased by about 0·4–0·6 pH units after two to three days of growth. pH of the culture solution was therefore readjusted every two days to 4·8 by addition of dilute solution of sodium hydroxide or sulphuric acid as needed. By this process, difference in the pH of two culture solutions was, as far as possible, eliminated. This pH change in the culture solution occurred up to boot stage of plant growth only and later, no necessity for readjustment of pH was felt. pH tests were done with E. Merck Special Indicator Paper No. 9555, having pH range of 3·8–5·4 in steps of 0·2 pH units.

All nitrogen levels, both for 20 and 100 p.p.m., were duplicated and additional pots were kept for study of uptake of nitrogen and of other nutrients up to ear-initiation stage. These latter pots were removed at this stage of growth on August 13 and plants were analysed for growth characters, total nitrogen, soluble nitrogen and mineral content. Mature plants, harvested on October 1, were also similarly analysed.

All nitrogen determinations were done by micro-Kjeldahl method. Soluble nitrogen was determined after dissolving the soluble fraction in 1% acetic acid and protein nitrogen was calculated by difference between total and soluble nitrogen.

EXPERIMENTAL RESULTS

(*a*) *Growth and crop yield.*—Growth characters at ear-initiation and at harvest of plants grown in ammonium and nitrate nitrogen are given in Table I.

TABLE I

Growth and dry matter weight of rice plant grown under ammonium and nitrate nitrogen

Date of sampling	Ear-initiation (13/8)				Harvest sample (1/10)			
Nitrogen source	Ammonium-N		Nitrate-N		Ammonium-N		Nitrate-N	
Nitrogen level p.p.m.	20	100	20	100	20	100	20	100
Top length cm.	104	102	100	108	134	135	144	152
Root length cm.	27	16	33	32	24	16	44	33
Tillers/pot (3 plants)	29	29	20	26	34·5	39·5	32·5	48·0
Ear No./pot (3 plants)	..	..	..	..	34·5	30·5	30·0	47·0
Dry matter weight gm./pot:								
Ear	..	..	..	..	55	31	54	81
Straw	30·8	23·7	16·7	24·1	55	44	39	75
Root	5·3	2·4	3·8	5·0	7·2	5·1	6·6	6·6
Total	36·1	26·1	20·5	29·1	117·2	80·1	99·6	162·6

At ear-initiation stage, highest top length of plant is obtained with nitrate nitrogen at 100 p.p.m.; there is, however, no highly marked difference in top length in various treatments. Root length, on the other hand, shows marked variation with treatments and plants grown on nitrate nitrogen, generally show a longer root than those grown on ammonium nitrogen. High level of ammonium nitrogen (100 p.p.m.) is seen to have deleterious effect on root development. Up to ear-initiation stage, tillering capacity is higher for ammonium nitrogen plants and total dry matter of root and shoot is highest with ammonium nitrogen at 20 p.p.m. Lowest dry matter weight is obtained with nitrate nitrogen at the same level.

At the harvest stage, however, nitrate nitrogen plants show much higher top length than the ammonium nitrogen plants. At moderate supply of nitrogen (20 p.p.m.), total tiller number and ear-bearing tillers per pot are

about the same for ammonium and nitrate plants. There is practically no difference in the ear weight of ammonium and nitrate nitrogen plants at this level but the total dry matter weight is more in the case of ammonium nitrogen plants, the increase being due to comparatively more straw yield. At 100 p.p.m., however, nitrate nitrogen plants put up considerably more tillers per pot than the ammonium nitrogen plants and highest dry matter weight of both ear and straw is obtained with nitrate nitrogen at this level. Ammonium nitrogen, at high nitrogen supply, appears toxic for plant growth and gives poor yield of both grain and straw. Plate X shows the general appearance of ammonium and nitrate plants as on September 20, about ten days before harvest. It is seen that top growth of the plant at 20 p.p.m. is about the same with ammonium and nitrate nitrogen, but root development is better with nitrate supply. At high levels of nitrogen (100 p.p.m.), both top growth and root development are benefited by nitrate nitrogen. High level of ammonium nitrogen is particularly deleterious for root growth, making the roots short, thick and fibrous with less root hairs and reduced surface area.

(*b*) *Nitrogen content.*—Nitrogen content (per cent. on dry matter basis) and total nitrogen absorbed as mg./pot by ear, straw and root at ear-initiation and harvest stages are given in Table II. Data show that both at ear-initiation and harvest stages, nitrogen per cent. in the plant at 20 p.p.m. nitrogen level is generally more in the nitrate nitrogen plants than in the ammonium nitrogen plants, except for straw at the harvest stage, where both treatments show almost the same nitrogen content. Total nitrogen absorbed per pot at this nitrogen level, on the other hand, is slightly higher in the ammonium nitrogen plants at both stages of growth. There is, however, practically no difference in nitrogen absorbed per pot between ear-initiation and harvest stages with two sources of nitrogen supply, at 20 p.p.m. nitrogen. At higher nitrogen level (100 p.p.m.), ammonium nitrogen plants generally show higher nitrogen content than nitrate nitrogen plants, both at ear-initiation and harvest stages, but total nitrogen removed per pot and also nitrogen absorbed between ear-initiation and harvest is considerably higher for nitrate nitrogen plants than for ammonium nitrogen plants.

Nitrogen metabolism of plant at ear-initiation and harvest stages, measured as the ratio of soluble to protein nitrogen in the straw, is shown in Table III. This ratio has been used by Tanaka *et al.* (1958 *b*) as a measure of efficient assimilation of absorbed nitrogen by rice plant. It is seen that at 20 p.p.m. nitrogen, there is not much difference in this ratio for ammonium and nitrate plants, both at ear-initiation and harvest stages. At higher nitrogen level (100 p.p.m.), this ratio is higher for ammonium nitrogen plants

TABLE II

Nitrogen content of rice plant grown under ammonium and nitrate nitrogen

Date of sampling	Ear-initiation (13/8)				Harvest sample (1/10)			
Nitrogen source	Ammonium-N		Nitrate-N		Ammonium-N		Nitrate-N	
Nitrogen level p.p.m.	20	100	20	100	20	100	20	100
N per cent.:								
Ear					1·44	2·56	1·58	2·08
Straw	1·61	3·08	2·16	2·95	0·80	2·72	0·76	1·76
Root	1·24	2·64	1·53	2·28	1·04	1·28	0·88	1·52
N absorbed mg./pot:								
Ear					792	794	853	1,685
Straw	496	730	361	711	440	1,197	296	1,320
Root	66	63	58	114	75	65	58	100
Total	562	793	419	825	1,307	2,056	1,207	3,105
N absorbed mg./pot between ear-initiation and harvest					745	1,263	788	2,280

TABLE III

Ratio of soluble to protein nitrogen of straw of rice plant grown under ammonium and nitrate nitrogen

Date of sampling	Ear-initiation (13/8)				Harvest sample (1/10)			
Nitrogen source	Ammonium-N		Nitrate-N		Ammonium-N		Nitrate-N	
Nitrogen level p.p.m.	20	100	20	100	20	100	20	100
Straw, total N %	1·61	3·08	2·16	2·95	0·80	2·72	0·76	1·76
Straw, soluble N %	0·41	1·07	0·48	0·61	0·17	1·16	0·20	0·32
$\frac{\text{Soluble N}}{\text{Protein N}^*} \times 100$	34	53	29	26	27	74	36	22

* Protein N is calculated as difference of total N and soluble N.

than for nitrate nitrogen plants and the increase in this ratio is particularly marked in ammonium nitrogen plants at harvest. Results show that while at moderate nitrogen supply, nitrogen assimilation keeps pace with nitrogen uptake irrespective of source of nitrogen supply, at high nitrogen levels, nitrogen metabolism in the ammonium nitrogen plants is disturbed resulting in higher accumulation of soluble nitrogen.

(*c*) *Mineral content of straw.*—Table IV shows the influence of nitrogen source on mineral content of straw at ear-initiation and at harvest. Mineral content of nitrate nitrogen plants is higher than that of ammonium nitrogen

TABLE IV

Mineral content in straw of rice plant grown under ammonium and nitrate nitrogen

(Per cent. on dry matter basis)

Date of sampling	Ear-initiation (13/8)				Harvest sample (1/10)			
Nitrogen source	Ammonium-N		Nitrate-N		Ammonium-N		Nitrate-N	
Nitrogen level p.p.m.	20	100	20	100	20	100	20	100
K_2O	2·50	2·35	4·15	3·95	2·50	2·65	5·75	3·60
CaO	0·63	0·36	0·97	1·02	0·39	0·37	1·12	1·71
MgO	0·29	0·17	0·45	0·55	0·15	0·10	0·26	0·53
Fe	0·05	0·03	0·02	0·02	0·02	0·02	0·04	0·04
Mn	0·02	0·01	0·08	0·09	0·03	0·01	0·08	0·14
P_2O_5	1·23	1·00	1·25	1·18	0·35	0·41	0·51	0·63
SiO_2	4·40	4·10	6·05	4·95	4·40	3·75	4·85	3·20

plants at both stages of growth. Nitrate nitrogen appears to accelerate the intake of potassium, calcium, magnesium and manganese. Phosphate intake is also accelerated by nitrate nitrogen but not to the same degree as that of basic ions. Manganese content of nitrate nitrogen plants is very high; iron content, however, does not show any constant trend, it is higher in ammonium nitrogen plants at ear-initiation and nitrate nitrogen plants at harvest. Silica content at harvest is also not much different in ammonium and nitrate plants.

(*d*) *Influence of change of nitrogen source at ear-initiation stage.*—In a supplementary experiment, influence of change-over of nitrogen source from ammonium to nitrate and *vice versa*, at ear-initiation stage was studied. Dastur and Malkani (1933) have reported that rice plant prefers ammonium nitrogen at early growth and nitrate nitrogen at later growth period. Tanaka *et al.* (1959) have shown that absorption process of nitrogen by rice plant is intimately related to the growth process and that nitrogen absorbed at certain periods during vegetative growth and ear development is most effectively utilized for production of grain. It was therefore thought that this preference for nitrogen source by rice plant may be connected with the growth phases of the plant.

Tables V and VI show the effect of change-over of nitrogen source at ear-initiation stage on plant growth and nitrogen content. At moderate levels of nitrogen supply (20 p.p.m.), change-over of nitrogen source from

TABLE V

Growth and dry matter weight of rice plant grown under ammonium and nitrate nitrogen

Nitrogen source ..	NH_4-NO_3*		NO_3-NH_4*	
Nitrogen level p.p.m. ..	20	100	20	100
Top length cm. ..	142	144	143	151
Root length cm. ..	31	27	33	28
Ear No./pot ..	31	60	30·5	33·5
Dry matter gm./pot:				
Ear ..	60	87	51	40
Straw ..	51	86	42	42
Root ..	9·0	8·9	5·9	6·0
Total ..	120·0	181·9	98·9	88·0

* Nitrogen source was changed at ear-initiation stage.

ammonium nitrogen to nitrate nitrogen or *vice versa* has practically no effect on top length, root length or ear-bearing tillers per pot. Nitrogen content of ear, straw and root is also not affected but straw shows a higher nitrogen content with ammonium nitrogen given after ear-initiation stage. Grain and

straw yield are, however, higher with ammonium nitrogen given at vegetative stage followed by nitrate nitrogen after ear-initiation.

TABLE VI

Nitrogen content of rice plant grown under ammonium and nitrate nitrogen

Nitrogen source ..	NH_4-NO_3*		NO_4-NH_3*	
Nitrogen level p.p.m. ..	20	100	20	100
N per cent.:				
Ear ..	1·68	2·08	1·68	2·48
Straw ..	0·80	1·76	0·96	2·08
Root ..	0·84	1·44	0·88	1·68
N absorbed mg./pot:				
Ear ..	1,008	1,810	857	992
Straw ..	408	1,513	403	874
Root ..	76	128	52	100
Total ..	1,492	3,451	1,312	1,966

* Nitrogen source was changed over at the ear-initiation stage.

At high level of nitrogen supply (100 p.p.m.), a considerable increase in the number of ear-bearing tillers per pot and grain and straw yield is obtained with ammonium nitrogen followed by nitrate nitrogen, later given at ear-initiation. Nitrogen content of ear and straw, on the other hand, is higher with nitrate nitrogen followed by ammonium nitrogen, but total nitrogen removed per pot is very much higher for treatment with ammonium nitrogen followed by nitrate nitrogen at ear-initiation stage, because of much higher crop yield obtained with this treatment. As previously shown, a high level of ammonium nitrogen at ear-initiation stage is again seen to have deleterious effect on growth and pulls down ear and straw yield considerably.

DISCUSSION

Rice plant grown under solution culture at moderate levels of nitrogen supply (20 p.p.m.) is seen to do equally well both under ammonium and nitrate nitrogen supply. Although ammonium nitrogen is seen to be a better source of nitrogen up to ear-initiation stage, both nitrogen sources

give about the same yield of ear at harvest. Straw yield, however, tends to be higher in ammonium nitrogen plants. At higher nitrogen levels (100 p.p.m.), nitrate nitrogen is decidedly better source of nitrogen supply for rice plant and ammonium nitrogen at this level has adverse effect on plant growth and root development. Nitrogen metabolism of high ammonium nitrogen plants is also disturbed, resulting in higher accumulation of soluble nitrogen to protein nitrogen. Tanaka *et al.* (1958 *b*) have earlier shown that higher ratio of soluble nitrogen to protein nitrogen in the plant tissue results in the malnutrition of the plant and pulls down grain yield considerably.

Asana (1945) has reported that it was not possible to grow healthy rice plant for long in ammonium culture at low pH of 4·7 and suggested that low pH, rather than ammonium nitrogen may be responsible for restricted root development. He has, however, used a high level of ammonium nitrogen (84 p.p.m.) in his trials. In the present study, healthy rice plants have been grown up to maturity at pH 4·8 with 20 p.p.m. ammonium nitrogen. At high level of ammonium nitrogen (100 p.p.m.), however, toxic effects on growth are observed. The failure on the part of Asana (1945) to grow healthy plants on ammonium nitrogen may, therefore, be attributed to high nitrogen level used in the trials rather than due to low pH of culture solution.

Nitrate nitrogen is seen to be superior to ammonium nitrogen, particularly in respect of uptake of other nutrients. Nitrate nitrogen promotes uptake of potassium and phosphate, both of which are metabolically absorbed by the plant. This shows that nitrate nitrogen helps promote plant respiration and metabolic activity, more than ammonium nitrogen. Results regarding high content of basic ions in the nitrate nitrogen plants are in conformity with those reported by Arnon (1939) for barley and Asana (1945) for rice. Phosphate content of nitrate plants, unlike that of barley (Arnon, 1939) is also higher than that of ammonium nitrogen plants.

More satisfactory source of nitrogen for rice plant seems to be ammonium nitrogen up to ear initiation stage followed by nitrate nitrogen at ear development. This change-over gives higher yield of grain and straw, both at 20 and 100 p.p.m. nitrogen supply. Ammonium nitrogen, particularly at high level, given at ear development stage is seen to be toxic for plant growth and pulls down grain yield. These findings are in general agreement with observations of Dastur and Malkani (1933), who suggested that rice plant, at early stages of growth, preferred ammonium nitrogen while at later stages of growth, it utilized nitrate nitrogen more effectively. Toxic effect of ammonium nitrogen on rice growth at later stages of development is also reported by Thelin and Beaumont (1934) who observed that up to

two months after transplanting, ammonium nitrogen gave higher weight of dry matter but at later stages of growth, nitrate nitrogen far surpassed ammonium nitrogen in total dry matter weight of plant.

Present study shows that this preference of source of nitrogen appears related to development phases of the plant and that ammonium nitrogen is suitable nitrogen source during vegetative stage for increasing tillering capacity of the plant and nitrate nitrogen is preferred by rice at the elongation and reproductive stages of plant growth. Dastur and Kalyani (1934) had suggested that this preferential uptake of nitrogen source at different stages of growth is due to the electrical charge on the protein of the cell, being on the alkaline and acidic side of iso-electric point at early and later stages respectively. The relationship of this preference for nitrogen source, with definite stages of plant growth may also be related to oxidation reduction character of the plant root. It has been shown by Arnon (1937) that assimilation of ammonium nitrogen by crop plants, unlike that of nitrate nitrogen is increased by relatively high oxygen supply in the nutrient medium. Roots of rice plants are reported by Mitsui (1955) to show oxidising character at early stages of development and strong reducing character at and after flowering. It may, therefore, be assumed that during vegetative growth period, comparatively higher oxygen tension prevails in the rhizosphere of rice plant, because of oxygen secretion by the roots and that this condition facilitates the uptake of ammonium nitrogen at this stage of growth. At the reproductive stage, as flowering approaches, there is low oxygen tension in the rhizosphere because of reducing character of root, resulting in restricted uptake of ammonium nitrogen and preferential uptake of nitrate nitrogen at this stage of development.

Summary

Comparative efficiency of ammonium and nitrate nitrogen source, for growth of rice has been studied under solution culture technique. It is seen that:—

(*a*) At moderate level of nitrogen (20 p.p.m.), ammonium and nitrate nitrogen are equally good nitrogen sources from the point of nitrogen uptake and grain yield but straw yield is higher with ammonium nitrogen.

(*b*) At high nitrogen supply (100 p.p.m.), nitrate nitrogen is superior to ammonium nitrogen as the latter gives restricted root growth and disturbed nitrogen metabolism in the leaf tissue resulting in higher accumulation of soluble nitrogen to protein nitrogen.

(*c*) Nitrate nitrogen is found to promote uptake of potassium, calcium, magnesium, manganese and phosphate by rice plant.

(*d*) Comparatively better growth and crop yield is obtained with ammonium nitrogen given up to ear-initiation and nitrate nitrogen at later growth. Ammonium nitrogen is, therefore, suitable during vegetative stage for increasing tillering capacity, while nitrate nitrogen is preferred by the plant at the elongation and reproductive stage. It is suggested that this preference for nitrogen source is related to the oxidation reduction character of the plant root at different stages of plant growth.

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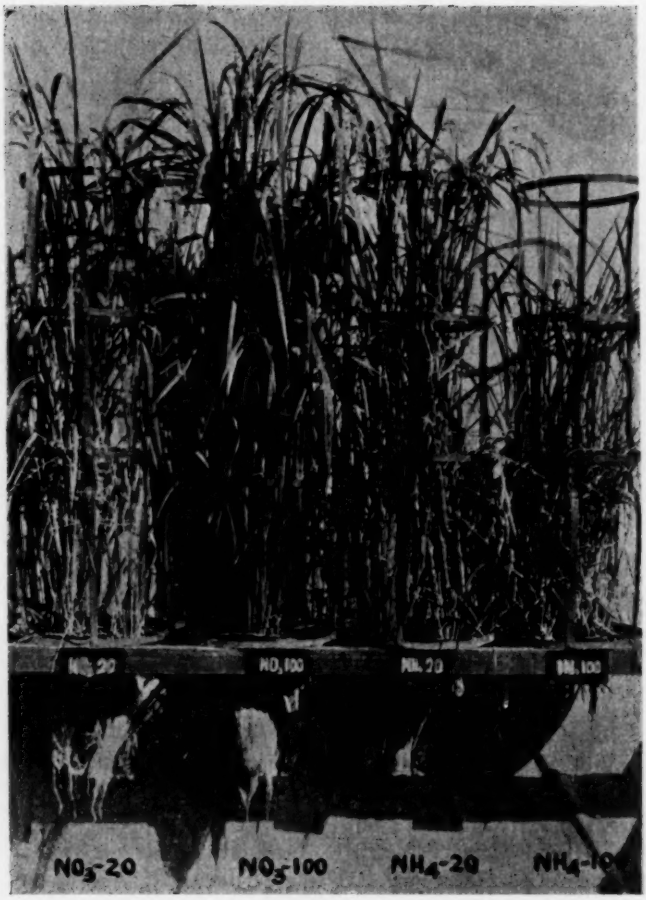

FIG. 1. Rice plants grown on ammonium and nitrate nitrogen at 20 to 100 p.p.m.

A NOTE ON THE FIRST PHYLLOSOMA OF *PANULIRUS BURGERI* (de HAAN)*

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Introduction

The authors started a few years back a survey of the Phyllosoma larvæ of the Indian waters (Prasad and Tampi, 1957). A thorough knowledge of these larvæ and the biology of the lobsters has now become essential because in recent years a fishery of considerable magnitude for lobsters has been established along the southernmost part of the west coast of India. The fishery is dependent on more than one species but at present off Vizhingam (76° 59′ 15″ E; 08° 22′ 30″ N), which is one of the important fishing centres, the dominant species constituting the fishery is *Panulirus burgeri*, popularly known as Burger's crayfish. It has been possible to obtain a berried specimen of this and the following account deals with the first Phyllosoma thus adding to the list of Phyllosomas of the Indian waters whose identity has been definitely established.

Description of the Larva

The berried specimen of *P. burgeri* was kept in a non-circulating seawater tank where the eggs hatched out. Although all the larvæ died in the course of two or three days, it has been possible to obtain and study the stages during hatching until the first Phyllosoma swims about freely. Gilchrist (1913) described for the first time a naupliosoma for the South African form of *Jasus lalandii*. The larva was an active swimming form with large biramous antennæ possessing long setæ. This moults after a few hours into the normal Phyllosoma. Feliciano (1956), who later described a prenaupliosoma stage in the development of *P. argus* remarked that further investigation is necessary to decide whether the prenaupliosoma and the naupliosoma stages occur within the egg or hatch as such. In the previous account (1957) the authors were not certain about the existence of a naupliosoma in *P. ornatus*. It may now be said with certainty that at least in *P. burgeri* neither a free living prenaupliosoma nor a naupliosoma, as described by some of the earlier workers in other forms, is present. However,

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the present authors have not studied the changes in the form of the developing embryos. It may be pointed out that the liberation of the Phyllosoma from the egg is more or less comparable to the process of hatching of the larvæ noticed in some of the other decapods (Prasad and Tampi, 1953). The larva that just emerges from the egg-capsule is unable to swim about as it remains completely rolled up inside another extremely thin and transparent membrane. At this stage the larva is soft bodied, the eyes droop laterally, the whole body as well as the appendages are curved ventrally and the setæ on the legs are only partly extruded so as to accommodate the larva within the delicate membrane. But the form of this larva is similar in all respects to that of the Phyllosoma except that it is not fully expanded. The larva sheds this membrane soon after the body and the appendages assume their turgidity. When once out of this covering the natatory setæ are fully extruded and the larva begins to swim about in the water. In view of these a separate name for this early soft-bodied larva does not seem warranted.

The first Phyllosoma of *P. burgeri* (Fig. 1 A) closely resembles the corresponding larva of *P. ornatus*, the only Phyllosoma of *Panulirus* of the Indian coast so far definitely correlated with its adult (Prasad and Tampi, 1957). It has been found that the differences in the first stage of Phyllosoma in these two species are only minor making specific distinction between them rather difficult. Nevertheless, it is possible to distinguish them on a detailed study and therefore the salient features of the larva of *P. burgeri* are compared with those of *P. ornatus* in order to help in differentiating them.

The Phyllosoma of both *P. ornatus* and *P. burgeri*, soon after hatching possess more or less the same type of orange-red chromatophores, particularly on the coxal segments of the pereiopods and between the base of the first and second antennæ, while those of the mandibular region and on the other segments of the appendages as observed in *ornatus* are wanting in *burgeri*. As these chromatophores seem to be mostly an early character and further as these contract or easily disappear on preservation, their significance in the systematics can only be very little.

The length of the first Phyllosoma of *burgeri* in these aquarium-hatched and preserved ones ranges from 1·35–1·40 mm., whereas that of *ornatus* averages 1·42 mm. A most noticeable difference between the two larvæ lies in the form and shape of the fore-body. Whereas in *burgeri* the length, maximum width and the length/width relation of the fore-body are of the order of 0·671 mm., 0·658 mm. and 1·02 the corresponding measurements in *ornatus* are 0·733 mm., 0·700 mm. and 1·05. Thus, in *burgeri*, the fore-body is comparatively smaller than that of *ornatus* but broader rela-

ve to their respective lengths. The change in the proportion is further accentuated by a distinct narrowing of the carapace just below the insertion of the second antennæ. Similarly, the proportion between the width of the fore-body and the hind-body is also slightly different in the two species; the hind-body/fore-body is 3/5 in the Phyllosoma of *burgeri* while it is $\frac{3}{4}$ in *ornatus*, the hind-body being relatively narrower in the former.

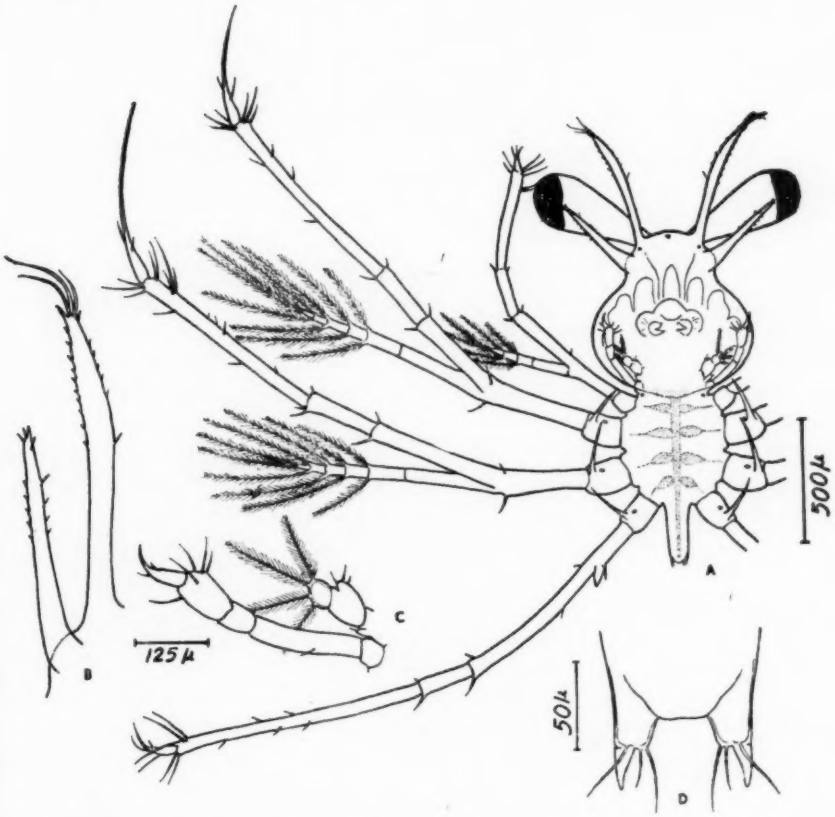

FIG. 1. A. The first Phyllosoma of *Panulirus burgeri*. B. The first and second antennæ. C. The second maxilla and the second maxillipede. D. The abdomen.

Considerable similarity between the larvæ of the two species is also evident in the shape of the eyes and their size in relation to the first antennæ, as well as the structure of the antennæ and other appendages. Figure 1 B shows the first and second antennæ of *P. burgeri*. Any difference in the

number of setæ or in their position seems to be insignificant, not to be too well relied upon as specific distinguishing characters. As in the Phyllosoma of *P. ornatus* the second maxilla has the last segment with the four setæ and also the three spines along the margin of the penultimate segment (Fig. 1 C). The first maxillipede is a very tiny, rudimentary bud (not figured in the earlier account by the authors), the second maxillipede is without an exopoditic segment while the third maxillipede is biramous bearing a setose exopodite characteristic of Palinurid Phyllosomas. The ventral coxal spines on the pereiopods are also very long and prominent, and similarly the pereiopods possess a spine at the distal end of their basipodite on the dorsal side adjacent to the beginning of the exopodites. The exopod of the third pereiopod, however, is only rudimentary as in *ornatus*. Johnson (1956) has described a spine in a corresponding position on the pereiopod in the larva of *P. gracilis* while it is said to be absent in the Phyllosoma of *P. interruptus*, thereby being able to distinguish the Phyllosomas of the two common species of spiny lobsters of the Californian coast. However, the presence of these spines seems to be a common characteristic of the larvæ of both *ornatus* and *burgeri*. It may further be added that the other spines on the legs do not seem to be very dependable as distinguishing characters. In trying to separate the two larvæ, the dactylus of the pereiopods seems to be more helpful in the present instance. While the general characteristics of the dactylus resemble in the two species the length and its proportion in relation to the propus (penultimate segment) show differences in the two species. The mean values are in Table I. The dactylus of the last pereiopod is much the same in the two larvæ. It is very much shorter than that in the first two pereiopods and is curved like a claw.

TABLE I

	P. ornatus	*P. burgeri*
Dactylus of first pereiopod ..	0·585 mm.	0·522 mm.
Dactylus/Propus ..	1·50	1·43
Dactylus of second pereiopod ..	0·872 mm.	0·736 mm.
Dactylus/Propus ..	1·11	1·21

The abdomen in both the species is narrow with parallel sides ending in two pointed tips each bearing three simple setæ (Fig. 1 D).

Small numbers of the early Phyllosoma of *P. burgeri* have been obtained in November, 1958 in the inshore plankton hauls taken off Vizhingam. In view of the wider distribution of this species their larvæ will undoubtedly be found in other regions. Descriptions of the later stages in the larval history of this form as well as other Phyllosomas of the Indian waters on which work is in progress will be published in due course.

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ON GASTRULATION IN CHELONIA

(*Geœmyda trijuga coronata* Anderson and *Lissemys punctata granosa* Schoepff)

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Introduction

The Chelonian blastula and its gastrulation agree in essential features with those of other Reptiles. Agassiz for the first time (1857) describes an embryonic shield in the Chelonia but details of development are not given. Mitsukuri and Ishikawa (1887) studied the germinal layers in *Trionyx* species. The process of gastrulation in *Emys lutaria taurica* is described by Mehnert (1891) and that of *Chelonia caouana* is worked out by Mitsukuri in 1894.

The present work is an attempt at a complete study of the Embryology of Chelonia in all details for which two species are chosen for investigation, *viz.*, *Geomyda trijuga coronata* and *Lissemys punctata granosa*. A *preliminary account* of the process of gastrulation and formation of the germinal layers is given in this paper; further detailed study of the development is in progress.

Material and Methods

Eggs of *Geœmyda trijuga coronata* and *Lissemys punctata granosa* were collected from natural surroundings and allowed to incubate under similar conditions. For the earlier stages, eggs were dissected out from females during the breeding season extending from November to January. The blastoderms are prepared by carefully opening one end of the egg and pouring out the yolk. Then the blastoderm is easily located since it remains attached to the shell, clearly visible against light. The portion of the shell with the blastoderm is cut out and the fixative poured into it. After a few minutes the shell is put in a bowl of fixative and the blastoderm separated.

Specimens were fixed in various fixatives like Bouin's fluid, Smith's fluid or F.A.A. fixative. Whole mounts were stained in borax carmine and cleared in Clove oil. Microtome sections were prepared in longitudinal and transverse series. Sections stained in mordant hæmatoxylin method gave the best results.

The Primary Blastula

The primary blastula of *Geœmyda trijuga coronata* (Photo 1) is similar in external features to that of other Chelonia. The area pellucida (*a.p.*, Photo 1) overlies a blastocœlic cavity and the embryonic shield (*e.s.*, Photo 1) which is rather eccentrically placed towards the caudal end is more ovoid than circular. The area opaca (*a.o.*, Photo 1) is distally situated and extends with the periblast syncytium over a large portion of the yolk. The embryonic shield has a median mass of cells at its caudal end. This special mass is the primitive plate (*p.p.*, Photo 1).

The Secondary Blastula

In subsequent development a secondary blastula is formed with an upper layer of epiblast and a lower layer of hypoblast. The epiblast in the embryonic shield is composed of columnar cells, four to five layers deep merging into the area pellucida as a single layer of flat cells. The hypoblast is but one cell thick and lines the underside of the embryonic shield from the limit of the posterior end of the primitive plate and is continuous with the periblast tissue. This layer (the cœnogenetic hypoblast), the paraderm of Kupffer (1882) arises by a process of proliferation and delamination from the primitive plate and the surrounding areas. As Pasteels (1937) finds there is also an *invagination* of endoderm cells from the superficial epiblast of the primary blastula. Wenckebach (1891) and Mehnert (1892) and Will (1892) are of opinion that there is an independent sheet of cells, below the primitive plate with which alone the lower layer of the surrounding parts becomes continuous. My observations confirm the views of Mitsukuri (1894) that the primitive plate becomes continuous with both epiblast and hypoblast at its own periphery.

Subsequent stages show that the epiblast of the middle area of the primitive plate is endodermal; the anterior half of the embryonic shield is composed of notochordal and neural organ forming cells. A small portion in between the endodermal and notochordal areas represents the prechordal plate.

Gastrulation

The phenomenon in *Geœmyda trijuga coronata* begins with a distinct *invagination* of the upper layer of epiblast in the anterior part of the median primitive plate area (*, Photo 2). The few cells invaginated pass downwards and forwards among the undifferentiated mass of cells of the primitive plate. From this stage there is a rapid increase of cell movements in the anterior part of the site of invagination. The epiblast of the presumptive

endodermal area (*ep.*, Photo 3) migrates backward causing a well defined pouch. Weldon (1883) finds that the passage arises at the front end of the primitive plate and Strahl finds it arising in the mid area of the plate. In *Geœmyda trijuga coronata* however the pouch arises in the anterior part of the middle primitive plate as illustrated in Photo 2.

The cell movements relating to the lower layers of epiblast in the vicinity of the site of invagination may be observed in Photos 4 and 5. Since the deepening of the archenteric pouch is effected by a process of *involution* more pronounced in the anterior boarder of the blastoporal pit, the lower layer of cells perhaps subjected to corresponding stresses also migrate according to the cellular configuration of the upper epiblast cells. Thus the lower epiblast cells involute commencing from the dorsal lip of the blastopore. An archenteron thus formed extends downwards and forwards towards the hypoblast along the antero-posterior embryonic axis (*ach.*, Photo 5).

The shifting forwards of the lower end of the notochordal canal (archenteron) is perhaps also caused by the forward growth of the primitive plate (*p.p.*, Photos 4 and 5). The invagination cavity in *Geœmyda trijuga coronata* and in *Lissemys punctata granosa* opens below into the subgerminal cavity (*v.o.*, Photo 4) in the mature gastrula. Regarding the extension of the cavity before it breaks through below Will (1890) states that in *Cistudo* and *Platydactylus* it is exactly co-extensive with the epiblastic embryonic shield. Mitsukuri (1894) finds that the cavity breaks through when it is quite small compared with the epiblast. It may be emphasised that in *Geœmyda trijuga coronata* and in *Lissemys punctata granosa* the notochordal canal opens below into the subgerminal cavity at a place not far anterior than the posterior one-third of the embryonic shield (*v.o.*, Photo 4).

The formation of a clear well defined ventral blastoporal opening (*v.o.*, Photo 4) is a result of the lower end of the canal reposing itself upon the primary endoderm (*en.*, photo 4). The cells in front of the compact peristomal mesoblast and those in the floor of the cavity degenerate and there appear numerous small openings. Eventually these form a single opening. The extreme anterior end of the invagination cavity is clearly distinguishable and does not take part in the formation of the above-said opening. As Mitsukuri finds the opening is confined only to its floor.

Meanwhile masses of cells delaminate from the roof of the archenteron and migrate forwards to particular positions within the gastrula. These movements are more pronounced in the notochordal and endodermal areas. The endoderm arises from the epiblast cells of the middle primitive plate area. As the invagination cavity extends downwards and forwards masses

of cells are proliferated from the invaginated endodermal material. A little before the archenteric pouch reposes upon the primary endoderm, cells proliferated from the floor of the archenteron, migrate forwards and downwards below the notochordal cells. These cells form a sheet of secondary endoderm below the median notochordal band. The endodermal material migrates inward as the initial dorsal lip is formed (*d.l.*, Photos 2 and 3) followed by the prechordal plate cells. Mitsukuri (1894) says he could not distinguish in Chelonia anything corresponding to the prechordal plate. In the mature Gastrula of *Geœmyda trijuga coronata* a prechordal plate composed of endodermal and mesodermal material is distinguished at the anterior end of the notochordal band in which position it lies below the presumptive neural plate (*pch.*, Photo 6).

The mesoderm is derived from materials primarily located in the primitive plate along its lateral boarders extending anteriorly on the sides of the notochordal and neural areas (*p.mes.*, Photo 3). At the beginning of the gastrulation synchronous with the invagination of the endoderm (*, Photo 2), cells from the presumptive mesodermal area migrate from the outer surface of the blastula and converge to the external margin of the blastoporal lip. These migrating cells may be located in surface stained blastoderms where cell movements are indicated by linear rows of cells starting from the peripheral primitive plate area and running in the direction of the blastopore. The mass of cells thus heaped up involute at the lateral lips of the blastopore (*l.lp.*, Photo 7).

However, most of the mesoderm of the anterior portion is produced by cell proliferation from the median invaginated band. Eventually a pair of mesoblastic bands (gastral mesoblast) are produced which are continuous, posteriorly with the mesoderm produced at the sides of and behind the blastopore (peristomal mesoblast). Anteriorly the prechordal plate mesoderm is derived from cells proliferated from the endodermal cells (*en.*, Photo 6) beneath. In the mature gastrula the mesodermal material extends into the extra-embryonic region and soon form the blood islands.

Summary

Gastrulation in *Geœmyda trijuga coronata* and *Lissemys punctata granosa* begins with distinct *invagination* followed by a process of *involution* and *cell migration*. The archenteric pouch is not co-extensive with the epiblastic embryonic shield and hence cell proliferation and migration are important factors in the formation of the primary germinal layers. The invagination of the endoderm cells is the first event in the initial process of gastrulation.

It is only after the endoderm material has completely invaginated that the presumptive notochordal cells migrate backwards preceded by the prechordal plate cells. A prechordal plate is seen in the mature gastrula at the anterior end of the notochordal band in which position it lies below the anterior end of the neural plate area. The mesoderm arises by involution of cells at the lateral lips of the blastopore and also by cell proliferation from the median notochordo-endodermal band. Posteriorly mesoderm is proliferated from the primitive plate. It is possible that the mesoderm of the prechordal plate is proliferated from the primary endoderm.

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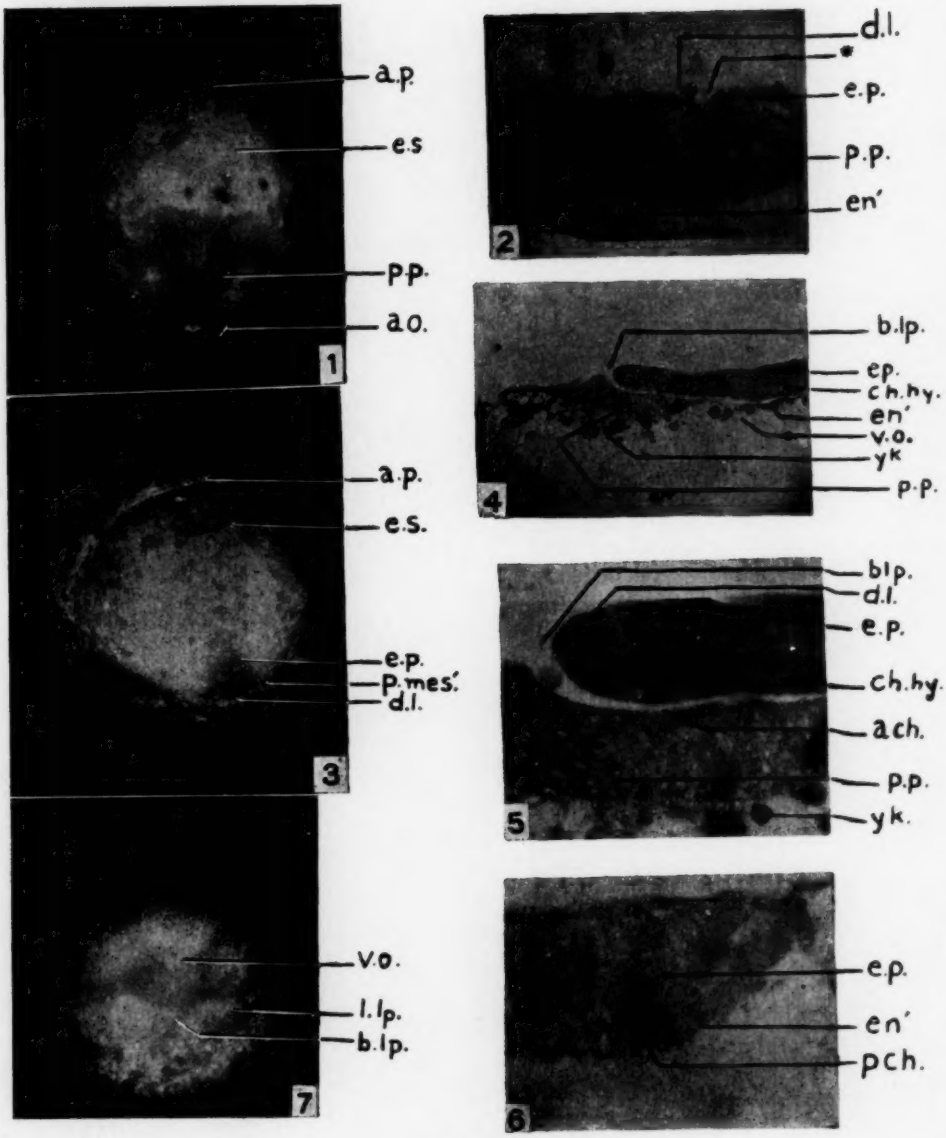
EXPLANATION OF PLATE XI

PHOTOS 1–7. Photo 1. The Blastoderm of *Geœmyda trijuga coronata*. Surface view of whole mount, ×80. Photo 2. Longitudinal Section of Blastoderm of G.t. coronata at the beginning


a.p.
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1
a.p.
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e.p.
p.mes.
d.l.
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l.l.p.
b.l.p.
7
d.l.
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e.p.
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6

PHOTOS 1–7

of gastrulation, ×320. The primitive plate area with the site of invagination is focussed (•). Photo 3. Gastrula of G.t. coronata in surface view, ×80. (The archenteron has not opened below. To compare with Photo 7, where the internal opening is visible). Photo 4. Median longitudinal section of the gastrula of G.t. coronata, ×80, showing the notochordal canal. Photo 5. Enlarged view of the blastopore and notochordal canal of Photo 4, ×320. Photo 6. Anterior portion of the gastrula in Photo 4 enlarged to show the prechordal plate, ×320. Photo 7. Surface view of the posterior end of the gastrula of G.t. coronata (whole mount) to show the internal opening of the blastopore, ×125.

Key to Lettering

a.p., Area pellucida; *ao.*, Area opaca; *ach.*, Archenteron (Notochordal canal); *blp.*, blastopore; *ch. hy.*, Chorda hypoblast; *d.l.*, Dorsal lip of the blastopore; *en.*, Primary endoderm; *e.p.*, Epibast; *e.s.*, Embryonic shield; *l.lp.*, Lateral lip of the blastopore; *p.p.*, Primitive plate; *pch.*, Prechordal plate; *v.o.*, Ventral (internal) opening of the blastopore; *y.k.*, Yolk.

ALTERATIONS IN RAT SERUM PROTEINS IN RELATION TO DIETARY PROTEINS AND B VITAMINS

I. Studies with Growing Rats

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The relation of dietary proteins to serum proteins in animals has been studied extensively. Differences in the rates of restoration of serum albumin, serum globulin and blood hemoglobin in depleted dogs and rats have been demonstrated as a result of feeding with different types of proteins (Pommerenke *et al.*, 1935; Melnick *et al.*, 1936; Weech and Goettsch, 1938; Madden and Whipple, 1940; Cox and Mueller, 1944; Seeley, 1945; Allison *et al.*, 1946; Robscheit-Robbins and Whipple, 1949), and with synthetic mixtures of amino acids (Robscheit-Robbins *et al.*, 1947; Sebrell and McDaniel, 1952). However, the influence of dietary protein quality on serum globulin fractions is not well defined. Chow *et al.* (1948) observed that enzymatic hydrolyzates of casein and lactalbumin were equally effective in restoring plasma albumin and γ-globulins but that casein was superior to lactalbumin in restoring β-globulins and fibrinogen, in protein depleted dogs. Damodaran (1953) compared the electrophoretic patterns of serum proteins of rats maintained on a 3 per cent. pulse protein diet with those of rats maintained on an 18 per cent. casein diet and observed that the former group had comparatively low concentrations of serum proteins although the relative concentrations of the different fractions were the same as those of animals on the casein diet.

There are indications that vitamins affect the serum protein profile. Greenberg *et al.*(1952) observed that hypoalbuminemia in monkeys in partial or complete deficiencies of vitamin B_6 and nicotinic acid was associated with a rise in γ-globulin level and, in choline deficiency, with elevated level of β-globulin. Similar observations were made by Fisher and Garrity (1953) who reported a decrease in albumin and increase in α- and β-globulins in sera of choline deficient rats.

Considerable data are available on antibody synthesizing ability of vitamin deficient animals (Axelrod and Pruzansky, 1955). Axelrod and coworkers (*see* Axelrod, 1953) have presented a rough classification of the effect

of 10 deficiency states in rats upon antibody production according to which severe, moderate and no impairment results from deficiencies, respectively, of pantothenic acid, pyridoxine and folic acid (PGA), riboflavin, thiamine, biotin, vitamin A and niacin-tryptophan, and vitamin B_{12} and vitamin D.

Since B vitamins in general and vitamin B_{12} and PGA in particular, have been implicated, through their roles in the metabolism of amino acids and nucleotides, in general protein metabolism, it was of interest to study the changes in rat serum proteins caused by (i) a general deficiency of B vitamins and, particularly, of vitamin B_{12} and PGA; and (ii) variations of dietary protein quality and level.

Experimental

Two different sets of experiments were carried out. The first one was aimed at ascertaining the influence of certain of the B vitamins on the utilization of amino acids from egg albumin and casein. Weanling male rats (Wistar strain) 40 g. in weight were divided into two groups of twelve each and maintained on purified diets with either devitaminised casein or egg albumin as sole source of protein at 18 per cent. level. Other additions (per cent.) were maize starch 72, arachis oil 3, shark liver oil 2, vitaminised sucrose 1, and salt mixture (U.S.P. XIV) 4. The arachis oil used was fortified with α-tocopherol and vitamin K (Menadione, Merck) such that 100 g. of the diet provided 12·5 mg. and 2·5 mg. respectively of the two vitamins. Each group was subdivided into two, receiving the B vitamins in the vitaminised sucrose at minimal and optimal levels as follows:

	Minimal	Optimal
	(mg./kg. diet)	
Thiamine hydrochloride ..	0·15	3
Riboflavin	0·15	4
Pyridoxine hydrochloride ..	0·15	3
Calcium pantothenate	1	10
Nicotinic acid	1	20
Inositol	50	200
p-Aminobenzoic acid	50	100
Choline chloride	50	200
Biotin	Nil	0·5
PGA	0·03	1
Vitamin B_{12}	Nil	0·15

It has been ascertained in this as well as in other experiments (Marfatia and Sreenivasan, in press) that throughout the experimental period of 8 weeks, none of the animals in the group receiving the low level of B vitamins shows any gross or clinical symptoms of deficiency of any single B vitamin. Animals were sacrificed after 8 weeks of *ad libitum* feeding.

In the second set of experiments, purified casein and defatted hot-alcohol extracted peanut meal were used as protein sources at 10 and 18 per cent. levels and the effects of PGA and vitamin B_{12} were studied. Young male rats, 50 g. in weight, were first depleted of their vitamin B_{12} and PGA reserves by feeding an iodocasein diet consisting of (in g./100 g. of the diet): vitamin-free casein 10, iodinated casein ('Protomone', Cerophyl Laboratories, Kansas City, Mo.) 0·15, arachis oil 6, shark liver oil 2, sucrose 9·85, maize starch 68, and salt mixture (U.S.P. XIV) 4. The sucrose provided the following vitamin levels (in mg./kg. of the diet): thiamine hydrochloride 6, calcium pantothenate 20, pyridoxine hydrochloride 6,r iboflavin 10, biotin 1, nicotinic acid 30, choline chloride 500, inositol 500, α-tocopherol 50, and vitamin K 10. These were considered adequate for the hyperthyroid condition.

At the end of 4 weeks, the animals were divided into groups of 6. One group was continued on the same diet modified by the omission of the iodinated casein and inclusion of 2 per cent. succinyl sulphathiazole. A second group was fed the casein at 18 per cent. level with adjustment against the maize starch. Two similar groups were with peanut meal at 10 and 18 per cent. protein levels. There were corresponding groups in each case receiving supplements of vitamin B_{12} and PGA at levels of 50 μg. and 5 mg. respectively per kg. of the diet. Besides these 8 groups, there were four additional ones with the casein or peanut protein diets, at 10 per cent. protein level, and with single additions of vitamin B_{12} or PGA at levels same as above. Growth was recorded over a five-week period of *ad libitum* feeding when the animals were sacrificed.

At the conclusion of the respective experimental periods the animals were anesthetised and, blood drawn from the inferior vena cava using a dry syringe was allowed to clot at 37° for one hour in sterile glass container. The separated serum was obtained by centrifugation at 2°. Livers were quickly excised and chilled in cracked ice.

Total serum protein was determined by the biuret method (Gornall *et al.*, 1949).

Paper electrophoresis.—Fractionation of serum proteins was carried out by electrophoresis on Whatman No. 3 paper strips, 5×34 cm., in a horizontal open-strip type cell with barbiturate buffer of pH 8·6 and ionic strength 0·075. Ten μl. serum was used and separation was effected at a constant current of 2·5 mA./strip for 16 hours at room temperature (28° C.). The subsequent procedure was as described by Jencks *et al.* (1955). The strips were dried in an air oven at 110° C. for 30 minutes and later stained for 16 hours in an aqueous bath containing 0·01 per cent. bromophenol blue, 5 per cent. glacial acetic acid and 5 per cent. zinc sulphate. Excess dye was removed by washing the strips in 2 per cent. glacial acetic acid solution, renewed at intervals of 5 minutes, for 20 minutes. A final rinse for 2 minutes in a solution containing 2 per cent. sodium acetate and 10 per cent. glacial acetic acid was followed by blotting and drying at 110° C. The strips were scanned after immersion in hot liquid paraffin, draining for 2 hours and blotting off excess paraffin. An 'EEL' scanner (Evans Electroselenium Ltd., Harlow, Essex) was used for the purpose.

Liver vitamin B_{12} was determined after liberation from the tissue by overnight incubation under toluene with papain (B.D.H., 25 mg./g. of liver) in 0·1 M acetate buffer of pH 4·6; the samples were then autoclaved, homogenized, neutralized and made to volume. Assays were carried out with *Lactobacillus leichmannii* (ATCC 7830) by a turbidimetric adaptation of the U.S.P. method (Rege, 1953).

Liver PGA was determined after autolysis in 0·2 M phosphate buffer of pH 7·6 using *Streptococcus fæcalis* R. (ATCC 8043) and the assay medium of Mitbander and Sreenivasan (1954).

Results

Food Intake and Growth

Although no pair-fed control animals were kept, the excess food intakes of the various controls over the corresponding experimentals were only 5–8 per cent. on high protein (18 per cent.) diets and from 15–20 per cent. on low protein (10 per cent.) diets. On the latter diets the consumption was 5–6 per cent. higher in the peanut protein group than in the corresponding casein group whereas no significant differences were observed between the intakes of egg albumin and casein and between those of casein and peanut protein when fed at 18 per cent. level. Food intakes at higher level of proteins were from 7–8 per cent. higher than at lower level.

The results presented below are as may be modified by these differences in food intakes. The differences in the intake of proteins being even smaller

TABLE I

Effect of variations in dietary protein quality and B vitamins on growth

In experiment 1, rats were reared for 8 weeks on purified diets of egg albumin or casein at 18 per cent. level with supplements of B vitamins either at optimal or at minimal levels. In experiment 2, rats deprived of their Vitamin B_{12} and PGA reserves were reared for 5 weeks on vitamin B_{12} and PGA free casein or peanut protein diets, at 10 or 18 per cent. protein level, with or without supplements of vitamin B_{12} and/or PGA.

The results are expressed as mean weight gains (g.) per week ± standard deviation.

Protein quality	Protein level %	Optimal/control	Minimal/deficient
Experiment 1:		B vitamins	
Egg albumin ..	18	18·2±3·2	15·4±2·0
Casein ..	18	20·0±2·6	12·0±1·8
Experiment 2:		Vitamin B_{12} and PGA	
Casein ..	18	42·6±3·8	36·7±4·6
Peanut protein ..	18	34·8±4·8	27·6±4·2
Casein ..	10	12·2±4·7	4·6±2·2
Peanut protein ..	10	14·0±1·9	3·2±2·8
		Vitamin B_{12}	
Casein ..	10		8·8±1·3
Peanut protein ..	10		9·1±2·4
		PGA	
Casein ..	10		6·4±3·1
Peanut protein ..	10		8·2±1·0

may not therefore be expected to modify the interpretation of the results reported.

The growth data for the two experimental series are summarized in Table I. Table II includes the liver levels of PGA and vitamin B_{12} in deficient and control animals of the second experimental series. It is seen that the improvement in growth resulting from optimal intake of B vitamins in experiment 1 is more marked in the casein than in the egg albumin group.

TABLE II

Vitamin B_{12} and PGA deficiencies

Liver levels of the vitamins in rats on deficient diets of casein or peanut protein at 10 or 18 per cent. protein level, with or without supplements of vitamin B_{12} and/or PGA.

Results are mean values for 6 rats ± standard deviation.

Group	Protein level %	Casein		Peanut protein	
		Liver PGA μg./g.	Liver vitamin B_{12} mμg./g.	Liver PGA μg./g.	Liver vitamin B_{12} mμg./g.
			Vitamin B_{12} and PGA		
Control ..	18	8·2±0·5	121·3±19·2	7·4±0·6	80·7±13·4
Deficient ..	18	3·0±0·7	35·9±21·4	2·1±0·9	14·5± 7·9
Control ..	10	6·8±0·6	92·6± 8·7	4·3±0·2	58·7± 9·8
Deficient ..	10	2·1±0·3	26·8±11·4	1·4±0·5	17·3± 4·2
			Vitamin B_{12}		
Deficient ..	10	5·4±0·5	29·7± 9·1	3·9±0·4	15·3± 4·2
			PGA		
Deficient ..	10	2·5±0·8	84·8± 7·2	1·8±1·1	60·3± 6·6

While growth retardation (Table I) and other deficiency manifestations (Table II) in single and combined deficiencies of vitamin B_{12} and PGA are as may be expected, the effects due to feeding of these vitamins are again more marked in the peanut protein than in the casein groups, particularly at lower intake of proteins. It is interesting to note that liver levels of the vitamins are higher in the casein-fed than in the corresponding peanut protein-fed groups.

Serum Proteins

The fractions labelled "Albumin" "α_1-", "α_2-", "β-" and "γ-" "globulins" are defined by the electrophoretic pattern illustrated in Fig. 1. In all cases, the significance of differences was calculated from data using individual pairs of results and evaluating "t". The corresponding "p" values were read from standard tables (Fisher and Yates, 1948) at the appropriate degrees of freedom. The differences were considered significant for values of "p" less than 0·05, and highly significant for values less than 0·01.

(*a*) *Relation to dietary proteins.*—Table III demonstrates the effects due to variations in dietary protein quality and level on serum proteins. Protein quality chiefly influences serum levels of albumin and α_1-globulin. Thus with egg albumin, the serum albumin level is higher than with casein

TABLE III

Influence of dietary protein quality and level on rat serum proteins

Figures represent the serum protein levels of rats fed on diets with optimal levels of B vitamins (experiment 1) and with PGA and vitamin B_{12} supplemens (experiment 2). For details of the experiments refer Table I.

Results are mean values for 6 rats with standard deviations.

Group	Protein level in diet %	Total protein	Albumin	Globulins			
				α_1	α_2	β	γ
				(g./100 ml. serum)			
Experiment 1:							
Egg albumin ..	18	6·14 ±0·16	2·42 ±0·06	1·06 ±0·03	0·57 ±0·04	0·99 ±0·04	1·09 ±0·09
Casein ..	18	6·24 ±0·07	2·25 ±0·05	1·29 ±0·07	0·65 ±0·03	1·03 ±0·07	1·01 ±0·09
Experiment 2:							
Casein ..	18	7·00 ±0·20	2·58 ±0·18	1·52 ±0·29	0·74 ±0·09	1·11 ±0·09	1·05 ±0·06
Casein ..	10	5·35 ±0·31	2·13 ±0·05	0·91 ±0·07	0·36 ±0·07	0·97 ±0·08	0·97 ±0·11
Peanut protein ..	18	5·90 ±0·47	2·30 ±0·23	1·15 ±0·05	0·66 ±0·08	0·95 ±0·04	0·84 ±0·07
Peanut protein ..	10	5·35 ±0·26	2·00 ±0·12	0·80 ±0·03	0·36 ±0·04	1·03 ±0·13	1·08 ±0·04

($p < 0·05$), and peanut protein is inferior to casein in this respect ($p < 0·05$). On the other hand, α_1-globulin concentration is higher in the casein-fed than either in the egg albumin-($p < 0·001$) or peanut protein-fed ($p < 0·001$) groups. The α_2-globulin level is also higher ($p < 0·05$) in the casein as compared to the egg albumin group whereas its levels in the casein and peanut protein groups are not appreciably different. β- and γ-globulin levels are higher ($p < 0·05$) with casein than with peanut protein, but only at 18 per cent. level, no significant differences existing in this respect at the lower level and between egg albumin and casein groups.

Except for an observed difference ($p < 0{\cdot}001$) between the 18 per cent. casein and peanut protein groups, protein quality, apparently, does not influence total serum protein concentration. On the other hand, there is lowering in total serum protein in low-level protein diets. This results from significant drops in levels of albumin, α_1-, and α_2-globulins, in the casein as well as in the peanut protein groups.

Variations in dietary protein quality, however, does not alter the relative distribution of serum protein fractions and although with low-level protein diets there is a relative lowering of α-globulins with rise in β- and γ-globulins, the albumin-globulin ratio remains unaltered.

(*b*) *Effects of B vitamins.*—A low intake of B vitamins causes a drop in total serum proteins, the extent of which is, apparently, influenced by dietary protein quality (Table IV). The depression is marked in the casein-fed rats ($p < 0{\cdot}01$), while those receiving egg albumin show a small but insignificant change. The reduction is largely confined to the γ-globulin fraction and is highly significant ($p < 0{\cdot}001$) for either group. In casein-fed rats there is, additionally, a marked drop ($p < 0{\cdot}001$) in α_1-globulin level.

(*c*) *Alterations in single and combined deficiencies of vitamin B_{12} and PGA* (*Table IV*).—There is a drop ($p < 0{\cdot}05$) in serum protein level in combined deficiency of vitamin B_{12} and PGA, especially on low protein diets ($p < 0{\cdot}01$). In the casein-fed groups, deficiency causes reductions in serum levels of albumin ($p < 0{\cdot}01$) and γ-globulin ($p < 0{\cdot}001$) at high protein intake. With lower intake, there are greater reductions ($p < 0{\cdot}001$) in serum levels of albumin, α_1-globulin and γ-globulin. In the deficient animals receiving peanut protein there is a marked reduction in γ-globulin level ($p < 0{\cdot}01$), especially with low intake of protein ($p < 0{\cdot}001$). This reduction is associated with appreciable lowering of albumin ($p < 0{\cdot}05$) and β-globulin ($p < 0{\cdot}01$) levels on low protein diet, and of α_1-globulin ($p < 0{\cdot}005$) level on high protein diet.

In vitamin B_{12} deficiency alone, there are marked reductions in albumin ($p < 0{\cdot}001$) and α_1-globulin ($p < 0{\cdot}01$) levels in the casein-fed rats, whereas, in the peanut protein-fed group α_2-globulin level is singly affected ($p < 0{\cdot}01$).

In single PGA deficiency, with casein in the diet, there is a decrease in albumin level ($p < 0{\cdot}01$) along with marked reductions ($p < 0{\cdot}001$) in β- and γ-globulin levels. In the peanut protein-fed rats, on the other hand, only the γ-globulin fraction is affected ($p < 0{\cdot}01$).

TABLE IV

Influence of B vitamins on rat serum protein fractions

Data indicate the deviations in the levels of serum protein fractions caused by (i) a minimal intake of all B vitamins (experiment 1), and (ii) single and combined deficiencies of vitamin B_{12} and PGA (experiment 2), under conditions where either the dietary protein quality or level or both are varied. For details of the experiments refer Table I.

Results are mean values for 6 animals ± standard deviation.

Group	Level of protein in diet %	Total protein	Albumin	Globulins			
				α_1	α_2	β	γ
				[Values as percentage of normal (control) groups]			
Experiment 1:				Minimal supplements of B vitamins			
Egg albumin ..	18	92·4± 7·8	91·4±5·6	106·6±7·8	108·7±7·8	93·0± 6·6	78·9±4·3
Casein ..	18	83·3±10·8	95·6±3·8	77·6±5·4	97·6±5·2	93·2±10·3	58·1±9·8
Experiment 2:				Vitamin B_{12} and PGA deficiency			
Casein ..	18	93·6± 3·1	86·9±7·2	96·0±5·0	101·3±3·3	110·9± 4·2	77·9±4·4
Peanut protein	18	91·6± 3·4	99·2±7·1	79·9±6·3	105·1±7·1	94·8± 5·1	81·4±6·8
Casein ..	10	77·8± 6·9	64·8±7·8	58·2±4·4	102·8±6·0	115·4± 6·1	78·4±8·3
Peanut protein	10	83·2± 4·6	89·6±6·4	98·8±7·2	97·3±4·3	86·3± 3·2	62·4±8·0
				Vitamin B_{12} deficiency			
Casein ..	10	86·7± 4·8	71·8±8·3	83·8±6·9	106·5±4·2	101·2± 9·3	105·1±3·3
Peanut protein	10	96·1± 5·4	93·0±3·3	104·1±9·8	84·2±6·1	104·2± 5·6	97·4±7·1
				PGA deficiency			
Casein ..	10	83·2± 4·3	83·9±4·3	93·3±6·9	102·2±9·3	73·2±10·2	70·1±5·9
Peanut protein	10	95·2± 6·9	95·9±7·8	96·9±5·4	91·3±5·6	103·2± 6·7	85·5±4·1

Peanut protein	10	95·2± 6·9	95·9±7·8	96·9±5·4	91·3±5·6	103·2± 6·7	85·5±4·1

Discussion

The observation that the response to growth with optimal levels of B vitamins is greater in the casein-fed than in the egg albumin group is also borne out from other studies (Marfatia and Sreenivasan, in press) relating to the utilization of dietary amino acids as influenced by B vitamins in general. The depression in serum protein level due to low intake of B vitamins is also greater in the casein than in the egg albumin group. This would suggest that with a superior protein higher levels of B vitamins do not exert an effect comparable to that with a low quality protein.

It is evident that dietary protein quality and level have profound influence on the serum proteins of the rat under normal and in stress conditions. Also, the effects due to these factors are interdependent, the influence of dietary protein quality on serum proteins being modified by protein level in the diet and *vice versa*.

The high values for serum albumin in rats fed egg albumin may be due to a similarity in the amino acid patterns in egg albumin and in serum albumin. The amino acid make-up of casein apparently favours synthesis of α-globulins. On the other hand, the imperfections in amino acid composition of peanut protein reflect upon serum levels of all the fractions, especially on those of albumin and α_1-globulin.

The observation that dietary protein qualitatively and quantitatively influences the serum levels of albumin and α_1-globulin would suggest a greater dependence of these fractions than of other fractions on dietary amino acids.

The constancy of the relative distribution of serum protein fractions in the face of variations in dietary protein quality is in conformity with the findings of Damodaran (1953), although it is seen that dietary protein level causes alterations in globulin fractions, the albumin-globulin ratio remaining unaltered.

The decrease in γ-globulin level with minimal intakes of B vitamins is similar to the impairment in antibody synthesizing ability of the rat known to result from specific deficiencies of certain of the B vitamins, notably, pyridoxine, pantothenic acid and folic acid (Axelrod and Pruzansky, 1955). Similarly, the observation that γ-globulin decreases in PGA deficiency but not in vitamin B_{12} deficiency is comparable with the impairment in antibody synthesizing ability of the rat in PGA deficiency and the absence of such impairment in vitamin B_{12} deficiency (Ludovici and Axelrod, 1951).

SUMMARY

The alterations in rat serum proteins resulting from (i) variations in dietary protein quality and level, (ii) minimal intakes of B vitamins and (iii) single and combined deficiencies of vitamin B_{12} and folic acid have been studied with egg albumin, casein and peanut protein diets at 10 and 18 per cent. protein levels.

Protein quality chiefly influences serum concentrations of albumin and α_1-globulin, the effects on other fractions being variable. Protein level affects serum levels of albumin, α_1-globulin and α_2-globulin. The effects due to these dietary variables are interdependent.

Variations in dietary protein quality do not alter the relative distribution of serum protein fractions. Changes in protein level cause shifts in the relative distribution of globulins, the albumin-globulin ratio remaining unaltered.

The nature and extent of changes in serum protein profile due to vitamin insufficiencies or deficiencies are also influenced, qualitatively and quantitatively, by dietary amino acids. With minimal intakes of B vitamins γ-globulin is chiefly reduced, but other fractions, particularly α_1-globulin, may also be affected. In a combined deficiency of vitamin B_{12} and folic acid a common reduction in γ-globulin is variably attended by reductions in albumin and in α_1- and β-globulins. In single vitamin B_{12} deficiency, the changes are confined to albumin, α_1-globulin and α_2-globulin fractions, whereas single folic acid deficiency chiefly affects γ-globulin with variable effects on albumin and β-globulin fractions.

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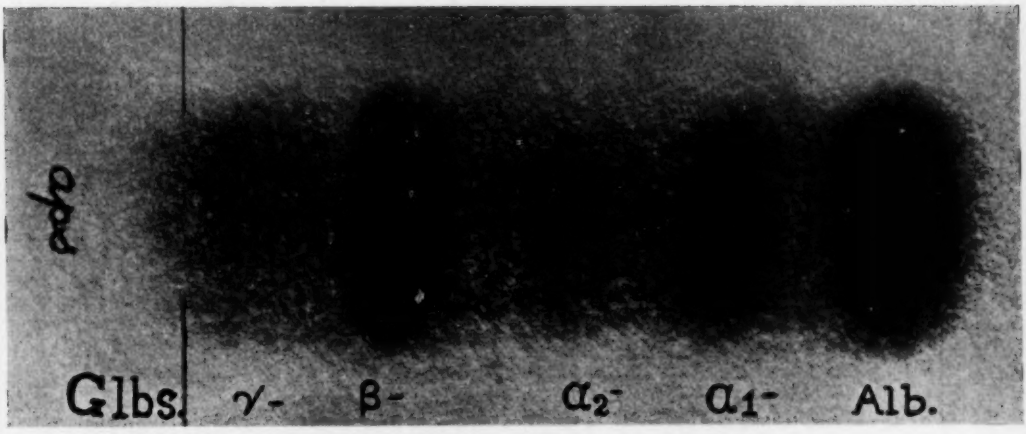

FIG. 1. A typical electrophoretic profile of rat serum protein fractions.

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THE DEVELOPMENT OF ENDOSPERM IN *LANTANA CAMARA* L.

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Introduction

A review of embryological literature on *Lantana* L. reveals that our knowledge of endosperm development in that genus is inadequate and is involved in ambiguity. Junell (1934) gave a brief comparative account of the formations of endosperm in three species—*L. camara* L., *L. involucrata* L., *L. chamædrifolia* Cham. In the succeeding year Patermann (1935) followed the development of chalazal endosperm haustorium in *L. trifolia*. Tatachar (1940) working on *L. indica* Roxb., refuted some of Patermann's observations and described a non-endospermous origin of the chalazal haustorium. According to him, no endosperm haustorium is differentiated at the chalazal end but instead the antipodal cells together organise themselves into a persistent haustorial apparatus. The observations of Crĕtĕ (1942) on *L. camara* are somewhat casual, brief and indicate no reference to Tatachar's work. According to Crĕtĕ, however, the chalazal haustorium is of endospermal origin. In regard to the micropylar haustorium of *Lantana* again, the descriptions are not uniform. Tatachar described a two-celled micropylar haustorium in *L. indica*, while according to Crĕtĕ (1942) the corresponding structure in *L. camara* is many-celled.

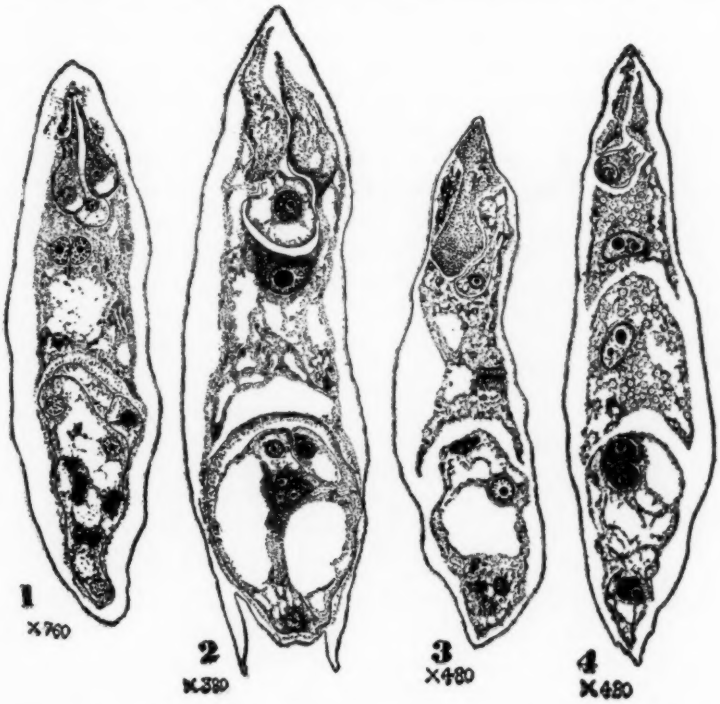
Material and Methods

Material for the present investigation was collected near Madras, and fixed in Formal Acetic Alcohol. Customary methods of dehydration and infiltration were followed. Sections were cut 8–12 μ in thickness and stained with Heidenhain's iron alum hematoxylin. Erythrosin in clove oil was used as counterstain.

Observations

The structure of the mature embryo-sac.—The mature embryo-sac consists of an egg apparatus, two juxtaposed polar nuclei and three multinucleate antipodal cells. Almost immediately after formation, the antipodal cells

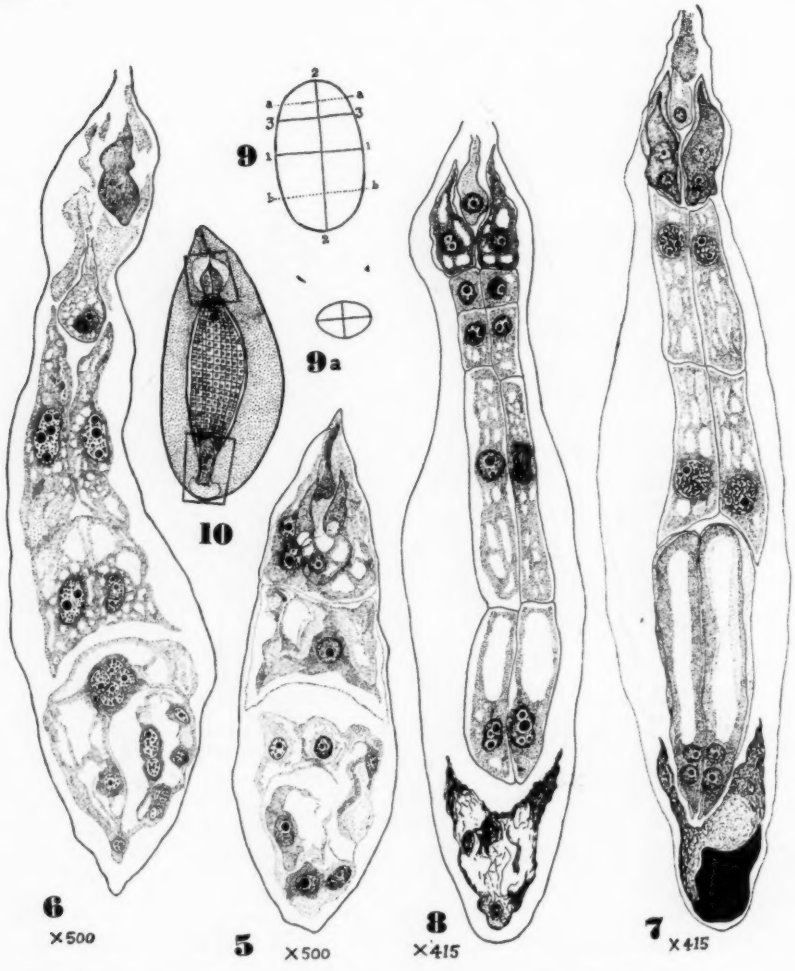
rapidly increase in size. The nucleus of each cell undergoes free nuclear mitotic divisions until six to eight nuclei are formed (Fig. 1). The coenocytic antipodal cells enlarge further so as to occupy nearly half or one-third of the vertical length of the embryo sac. It may be noted incidentally that the divisions of the nucleus of the antipodal cell need not strictly follow a simultaneous sequence; not only the divisions of certain nuclei may be accelerated



TEXT-FIGS. 1–4. Fig. 1. Mature embryo-sac. Fig. 2. Embryo-sac after double fertilization showing the oospore, the primary endosperm nucleus and the antipodal unit. Fig. 3. First division of the primary endosperm nucleus. Fig. 4. Two-celled endosperm; starch grains are abundant in the cells.

or retarded, but also some of the nuclei fail to divide. This situation explains the more or less fluctuating number of nuclei in the coenocytic antipodal cells. The antipodal unit begins to show signs of degeneration after four endosperm cells are formed (Fig. 6) and undergoes a somewhat rapid disintegration (Fig. 7). During or prior to degeneration the membranes of the antipodal cells often break down or collapse and the protoplasts of the cells in question merge with one another. At the stage when the endosperm haustoria are

well differentiated, the antipodal unit is completely absorbed and dark remains of its mass can be observed at the chalazal end of the embryo-sac (Fig. 7).



TEXT-FIGS. 5–10. Fig. 5. Three-celled endosperm. Fig. 6. Four-celled endosperm. Fig. 7. Differentiation of haustoria. Fig. 8. First few divisions in the middle tier of endosperm cells. Only two cells of the haustoria are shown. Fig. 9. Diagrammatic representation of the sequence of the planes of division of the primary endosperm nucleus. Fig. 9 *a*. Cross-sectional view of the micropylar and chalazal tiers of endosperm cells at levels marked *aa* and *bb* in Fig. 9. Fig. 10. Diagram of an young seed in longi-section showing haustoria, endosperm and embryo.

Endosperm

The first division of the primary endosperm nucleus (Fig. 3) is followed by the laying of a transverse wall which divides the embryo-sac into the primary micropylar and chalazal chambers (Fig. 4). Next, the micropylar chamber undergoes division by a vertical wall (Fig. 5). The chalazal chamber divides in the same plane and thus four endosperm cells in two tiers of two cells each are formed (Fig. 6). The two micropylar cells divide by transverse walls. This leads to a three-tiered group of endosperm cells each tier consisting of two cells—the micropylar tier, the middle tier and the chalazal tier. The micropylar tier differentiates as the micropylar haustorium and the chalazal tier as the chalazal haustorium. The middle tier by successive divisions gives rise to the cellular endosperm. The two cells of the micropylar haustorium soon undergo division by vertical walls so as to form a four-celled tier of more or less elongated cells (Fig. 7). These cells are aligned in a tetragonal configuration, which pattern remains undisturbed during later development (Figs. 7, 9 *a*, 11). Similar vertical division takes place in the cells of the chalazal tier also, resulting in a similar configuration of the four constituent cells (Fig. 7).

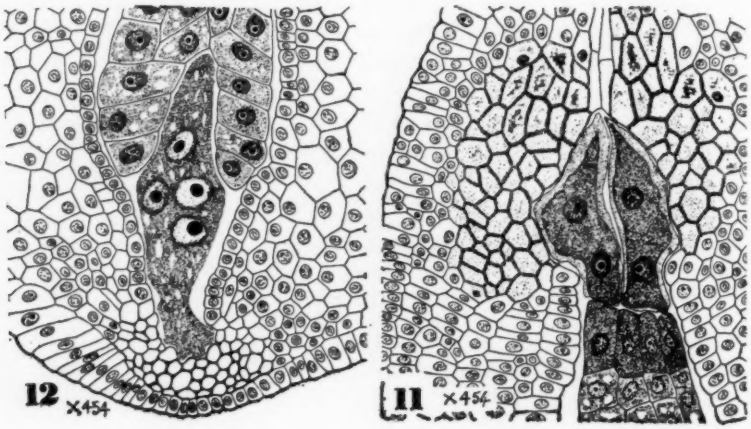
The sequence of the early planes of division of the primary endosperm nucleus are represented in Figs. 9, 9*a*.

The early few divisions in the middle tier are predominently in transverse plane (Figs. 7, 8), so that the body of the endosperm keeps pace with the elongation of the embryo-sac. Subsequently, however, intercalary divisions set in indiverse planes, thereby increasing in volume (Fig. 10).

The cells of the micropylar haustorium remain uni-nucleate and their free ends assume a more or less tapering appearance (Fig. 11); the cells remain distinct from each other during later stages also, after assuming a more or less falcate contour (Fig. 11). A few layers of endosperm cells lying just below the micropylar haustorium also get deeply stained (Fig. 11). They do not, however, show any change either in size or in shape; or in morphological appearance characteristic of endosperm haustoria. It is doubtful whether these cells function as accessory haustoria.

As has been stated already, the chalazal haustorium is composed of four uni-nucleate cells whose alignment is similar to that of the micropylar haustorial cells. The component cells of the chalazal haustorium are more or less cylindrical with their lower ends tapering (Figs. 7, 8). All the four cells remain uni-nucleate and each of them shows a cylindrical vacuole in the upper part (Figs. 7, 8) while the nucleus occupies the chalazal extremity

of the cell. In late stages the separating walls dissolve, the vacuoles disappear, and complete cytomyxis is accomplished. In longi-sections of young seeds the composite chalazal haustorium appears like a spindle-shaped structure with both ends somewhat tapering (Figs. 10, 12). Even though the lower



TEXT-FIGS. 11–12. Fig. 11. Enlarged view of micropylar haustorium and the surrounding regions marked by a square in Fig. 10. Fig. 12. Enlarged view of chalazal haustorium and the surrounding regions marked by a square in Fig. 10.

end of the chalazal haustorium shows some tendency towards the formation of short lobes, no conspicuous branches are produced. The endosperm cells bordering upon the chalazal haustorium get stained deeper and are comparable to similar cells at the micropylar end (Fig. 12).

DISCUSSION

The nature of the antipodal set-up in the genus *Lantana* needs a comment. A critical perusal of available literature indicates that the usual condition in the family Verbenaceæ appears to be an early degeneration of the antipodal cells. However, in a few genera like *Lantana* (Junell, 1934; Tatachar, 1940), *Clerodendron* (Misra, 1939) they not only persist but also undergo divisions. Junell's (1934) account is rather brief and ambiguous in the sense that he interpreted this feature as being due to the persistence of megaspores (or of their derivatives), a conclusion that is not borne out by evidence. Tatachar's (1940) account clarifies the issue to some extent. According to this author, no megaspores are involved but the nuclei of the antipodal cells themselves undergo two or three free nuclear divisions. That this is so is also confirmed in the present investigation. In contrast to the situation in

Lantana, Clerodendron exhibits actual division of the antipodal cells themselves so that after full development, a group of twenty cells constitute the antipodal apparatus (Misra, 1939).

Tatachar (1940) believed that the three coenocytic antipodal cells in *Lantana* constitute the chalazal haustorium. In his own words, "the three enlarged multinucleate antipodals together seem to form a very aggressive haustorium. Finger-like protrusions directed from them enter the chalazal tissue and their activity is clearly indicated by the degenerating cells of that region."

The sequence of endosperm development described here for *Lantana camara* L. differs in a significant way from the account given by Tatachar for *Lantana indica* Roxb. In the latter the endosperm does not build haustorial structures at the chalazal end. On the other hand, the antipodal unit is said to persist and function as an aggressive haustorium, at the chalazal end of the ovule. In *L. camara* not only an antipodal apparatus similar to the one described by Tatachar has been demonstrated but also the organisation of chalazal endosperm haustorium which is totally independent of the antipodals. Even after the initiation of the chalazal endosperm haustorium, the degenerating antipodal apparatus is clearly seen in every preparation (Figs. 7, 8). Thus, at least in *L. camara*, a normal chalazal endosperm haustorium, as in other members of the family, is organised.

A critical appraisal of Tatachar's (1940) illustrations of *L. indica* indicates the possibility of a different interpretation than the one given by him. It may be mentioned at the outset that Tatachar's Figs. 5–9 correspond in essence to Figs. 1, 2, 12, 5 and 7 respectively (of the present text). A comparison of these two sets of figures indicates the similarity between the two plants. The similarity becomes much clearer if Fig. 4 of the present paper is interpolated as Fig. 7 between Figs. 6 and 8 in sequence of Tatachar's illustrations. It is obvious that Tatachar mistook the more or less persisting antipodal apparatus for the chalazal haustorium. Also, he appears to have missed the complete sequence of the early stages. For example, his Fig. 8 (which corresponds to Fig. 5 of the present text) clearly indicates the initial cell of the chalazal endosperm hasutorium being superposed on the antipodal apparatus. Again in Fig. 9 (corresponding to Fig. 7 of the present text) the blocking out of the endosperm haustoria could be seen. Thus in Fig. 9, the cell tiers from the chalazal end are: (*a*) antipodal apparatus, (*b*) two-celled chalazal endosperm haustorium, (*c*) the middle tier of two cells which by subsequent divisions builds the body of the endosperm and (*d*) the two cells of the apical tier constituting the initials of the micropylar

haustorium. Thus *L. camara* agrees in all essentials with *L. indica* so far as the development of chalazal endosperm haustorium is concerned. The actual number of cells constituting the chalazal haustorium in *L. camara* was not recorded by Crĕtĕ (1942). He described a binucleate chalazal haustorium whereas the present work indicates the actual number of cells involved.

Although Crĕtĕ (1942) figured a four-celled micropylar haustorium in *L. camara*, in the text he stated that it is composed of many cells. He also agreed with Junell (1934) in regard to the haustorial role of all the constituent cells. The present investigation has revealed that the micropylar haustorium in *L. camara* consists of only four cells at maturity. One point of difference between the present account and that of Crĕtĕ lies in the configuration of the four cells of the micropylar haustorium. Crĕtĕ's figure indicates that it consists of two tiers of cells while in my material the cells are observed to lie consistently in a single tier.

It must be remarked that the endosperm cells subjacent to the micropylar haustorium also possess great avidity for stains and in the preparations the cells appear darkly or densely stained as those of the haustoria. This situation is reported in other species of *Lantana* also. As far as I am able to make out, these cells are not the derivatives of the micropylar haustorium. In view of this the statements of Junell (1934) and Crĕtĕ (1942) that more than four cells are involved in the micropylar haustorium and that the accessory cells are also functionally haustorial (by virtue of staining reactions) is to be looked upon as being unproven.

The presence of a zone of integumentary cells with ' reticulate ' thickenings on their walls, around the micropylar haustorium was recorded for *Stachytarpheta indica* by Tatachar (1940). A similar record for *Lippia nodiflora* was made by Maheshwari (1954). A zone of such integumentary cells has been observed in *L. camara* by Crĕtĕ (1942). The present investigation confirms this. The wall structure of the concerned cells here, however, is not due to reticulate thickenings but due to the presence of minute, closely spaced simple pits. Thus the individual cells belong to the category of selereids or stone cells.

SUMMARY

The multinucleate antipodal cells in *Lantana camara* L. are described with reference to their duration. The occurrence of mitotic divisions of the antipodal nuclei is confirmed. The antipodal unit begins to degenerate after the second cell generation in endosperm.

The pattern of mature delineation of endosperm is blocked out when the endosperm is composed of three superposed cells. The micropylar cell undergoes two divisions at right angles to each other to constitute a four-celled micropylar haustorium. The chalazal cell differentiates in a similar way into the chalazal haustorium. The middle cell builds up the body of the endosperm. The four cells of the micropylar haustorium remain as such at maturity while cytomixis is accomplished in the chalazal haustorium, at later stages. The reported occurrence of an antipodal haustorium is disproved.

ACKNOWLEDGEMENT

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